THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. XLVII—No. 1
Issued September 1, 1918

BALTIMORE, U. S. A.
1918

Entered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorised on July 5, 1918.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 47 SEPTEMBER 1, 1918 No. 1

THE ACTION OF PITUITARY EXTRACT ON THE KIDNEY

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Received for publication June 11, 1918

The essential contribution of this paper is a study of the changes in the blood flow and the oxygen consumption of the kidney during the diuresis evoked by pituitary extract. This was done in an endeavor to throw further light on the mode of action of the extract on the kidney, a question to which conflicting answers have been given. These contradictions result from the dual nature of the kidney mechanism and the complex circulatory conditions obtaining in this organ which make it difficult to explain results on the basis of vascular and volume changes.

Magnus and Schäfer (1) and Schäfer and Herring (2) were the first to recognize and investigate the diuretic action of extracts of the pituitary body. They explained the diuresis as due to direct stimulation of the renal epithelium. Their conclusion was based on the lack of parallel between the diuretic effect on the one hand and the blood pressure and kidney volume on the other hand. For a discussion of the data upon which this conclusion was based the reader is referred to Schäfer's recent review of the subject (3). According to his conception, the active diuretic principle bears the same relation to the kidney that secretin bears to the pancreas. Hoskins and Means (4) have supported Schäfer's view. An opposite conclusion has been arrived at by Houghton and Merrill (5) and by King and Stoland (6). From a comparison of the blood pressure, kidney volume and urine flow they consider that the vascular changes following the injection of pituitary extract are sufficient to explain the diuresis. Pal (7) and later Cow (8) working with isolated arterial rings, after the method of Meyer, found

a specific dilator action on the renal vessels. McCord (9) found that the vessels of the isolated perfused kidney were dilated by pituitary extract while the vessels of other organs (e.g., spleen) were constricted.

In view of these conflicting opinions it seemed to the authors that a study of blood flow and oxygen consumption, using the method of Barcroft and Brodie (10), might throw further light on the question. Using this method, Barcroft and Straub (11) showed that under certain conditions (e.g., injection of Ringer's solution) well-marked diuresis may be induced without any change in oxygen consumption by the kidney. The diuresis induced by other means (e.g., urea injection) was accompanied by a decided increase in oxygen intake. It was considered therefore that, if the diuresis induced by pituitrin was associated with an increase of metabolic activity as indicated by the oxygen intake, this would furnish additional confirmation of Schäfer's view, while on the contrary if no change in metabolic activity was found, it would be reasonable to consider the process purely physical.

EXPERIMENTAL

The experiments were mainly on cats. The animals were anaesthetized with urethane supplemented by ether when necessary. The methods of dissection, collection of the blood and measurement of its rate of flow were similar to those previously described by Barcroft and Brodie (10). In a few experiments on pregnant cats, evisceration was avoided by using the greatly enlarged ovarian vein for the measurement of the blood flow. In this way the nerve supply to the kidney was preserved intact. This method was suggested to one of us by Mr. Barcroft. Urine was collected by means of a bladder cannula and the flow recorded by a drop-counter. Oxygen determinations were made with the Barcroft blood-gas apparatus, using the differential method described by Barcroft (12).

The preparations used were pituitrin (Parke, Davis & Company) and infundin (Burroughs Welcome). In experiments where the two preparations were used alternately a greater preliminary fall of blood pressure was noted with the infundin, indicating perhaps a slightly greater content of accompanying vasodilator substance. Otherwise the action of the two preparations appeared identical.

In general the results we obtained were in conformity with those of Schäfer and his coworkers. The intravenous injection of pituitary extract was commonly followed by an initial fall of blood pressure.

Apparently this fall was peripheral in origin rather than cardiac as it appeared as promptly when the injection was made directly into the arterial system after the method devised by Mathison (13). This brief

TABLE 1

*Effect of repeated injections of pituitary extract on blood pressure, urine flow and blood flow**

Experiment	Injection number	Time interval	Blood pressure (carotid)		Urine flow (drops per minute)		Blood flow (cubic centimeters per minute)		Remarks
		Minimum	Before	After	Before	After	Before	After	
A	1		126	150–124	3	17			Infundin 0.25 cc. in 5 cc. saline
	2	30	110	120–100	11	16			Infundin 0.25 cc. in 5 cc. saline
	3	30	116	120	12	41			Infundin 0.25 cc. in 5 cc. saline
	4	30	108	120	11	34			Pituitrin, 0.5 cc.
	5	30	116	128	8	22			Pituitrin, 0.5 cc.
	6	30	118	120	12	17			Infundin, 0.25 cc.
B	1		162	186–174	12	24			Pituitrin, 0.5 cc. in 2 cc. saline
	2	15	165	178	18	27			Pituitrin, 0.5 cc. in 2 cc. saline
	3	15	160	172	14	24			Infundin, 0.25 cc. in 4 cc. saline
	4	15	140	186	14	24			Infundin, 0.25 cc. in 4 cc. saline
	5	15	140	148	9	13			Pituitrin, 0.5 cc.
	6	15	146	160	11	14			Infundin, 0.5 cc.
	7	15	144	148	8	14			Infundin, 0.5 cc.
	8	15	142	142	8	16			Pituitrin, 1 cc.
C	1		152	188	6	16	27.9	37.8	Pituitrin, 1 cc.
	2	15	152	164	4	27	29.2	48.5	Pituitrin, 1 cc.
	3	20	154	160	7	27	34.6	44.4	Pituitrin, 1 cc.
	4	20	142	142	16	30	44.4	55.8	Pituitrin, 1 cc.
	5	15	140	130	14	23	45.2	56.6	Pituitrin, 1 cc.
	6	20	120	120	7	17	44.5	46.5	Infundin, 0.5 cc.

* The measurements of blood pressure as given in the tables were taken just at the time when the blood flow was measured. They therefore do not always indicate the maximum point reached as a result of the injection. Our plan was to take the blood flow and samples for analysis at the point when urine flow seemed at its maximum.

initial fall was followed by a rise which persisted for a variable time. Associated with these circulatory changes there was first suppression or diminished secretion of urine coinciding with the fall of blood pressure. Diuresis as a rule began just beyond the crest of high pressure. Our observations also confirm the statement of Magnus and Schäfer, and Schäfer and Herring that the diuresis may outlast the rise of blood pressure or may be copious with a fall.

Repeated injections gave, in general, diminishing effects on the blood pressure so that after the first two or three injections the initial fall was the prominent feature and the after-rise often failed to reach the earlier average. With few exceptions the diuretic effect also tended

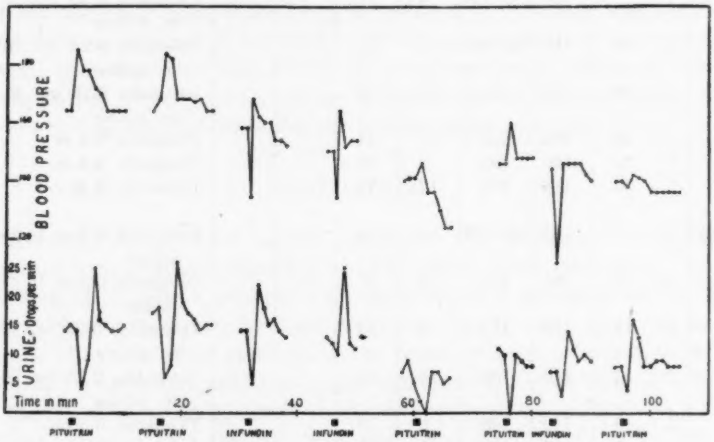

Fig. 1

to diminish with repeated dosage. This diminution of diuretic action was not as definite nor was it parallel with the lessening circulatory effect.

By referring to table 1 the reader can compare the above described changes in detail. It may be seen that in experiments A and B the maximum rise of blood pressure followed the first injection while the maximum urine flow and blood flow through the kidney came subsequent to the second and third injections respectively. In experiment C the maximum blood pressure change and maximum urine flow approximately coincided. In figures 1 and 2 the results of repeated injections are presented graphically.

In table 2 the effects of pituitrin on blood pressure, blood flow through the kidney and diuresis are compared in greater detail. It is seen that whether the arterial blood pressure rises or falls, increased flow of blood through the kidney is a constant accompaniment of diuresis. In a general way the two effects run parallel, a free diuresis being associated with a decided increase in blood flow. In a number of instances the quantity of blood flowing through the renal vessels per minute was doubled. We noticed repeatedly that following the injection of the pituitary hormone, the venous blood was arterial in color owing to the rapidity of the flow. In some instances in which but little diuresis

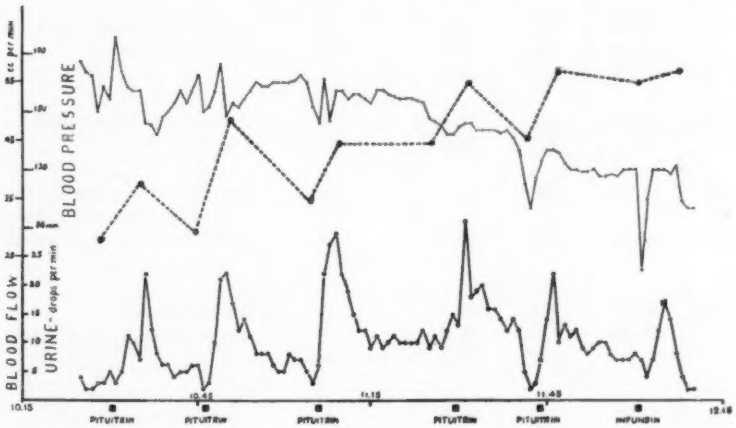

Fig. 2

could be induced, the blood flow also showed but little change even though the blood pressure was raised.

Data concerning the influence of pituitrin on the oxygen consumption by the kidney are given in table 3. It may be seen that totally independent of the extent of the diuresis induced, the amount of oxygen used by the kidney remained uninfluenced by the pituitary hormone. In no successfully completed experiment was there evidence that pituitrin caused increased work on the part of the renal cells as measured by their oxygen intake.

In several instances as a check on our method we compared the effect of the pituitary extract with that produced by urea or sulphate (table 4 and fig. 3). In sharp contrast to pituitrin, the urea and sodium sul-

TABLE 2

Effect of injections of pituitary extract on blood pressure, blood flow and urine flow

EXPERIMENT	TIME	BLOOD PRESSURE MM. HG. (CAROTID)	BLOOD FLOW THROUGH KIDNEY (CUBIC CENTIMETERS PER MINUTE)	URINE (DROPS PER MINUTE)	REMARKS
1	10.40	180	17.64	Occasional drop	Animal not eviscerated
	11.16	182	36.54	11	After 0.5 cc. pituitrin
	11.35	142	23.76	7	
2	11.00	138	48.0	6	Animal not eviscerated
	11.21	124	59.4	10	Pituitrin, 1 cc.
	11.59	112	48.0	6	
3	10.34	166	36.8	9	Animal not eviscerated
	11.37	140	90.0	20	Pituitrin, 1 cc.
4	11.50	138	34.0	0	Animal eviscerated. Urine flow from one kidney
	12.21	140	66.0	10	Pituitrin, 2 cc. (two injections, 1 cc. each)
5	10.33	154	22.3	6	No evisceration
	10.50	146*	33.9	13	5 min. after 1 cc. pituitrin
	11.35	122	27.6	Occasional drop	
6	11.03	158	23.0	0	Animal eviscerated
	11.15	154	32.3	5	5 min. after pituitrin
	12.20	100	21.0	0	
	12.25	118	23.0	5	After 2nd injection, 1 cc.
7	10.47	148	19.0	0	Animal not eviscerated
	11.10	144	37.5	2	1.5 cc. pituitrin
	11.58	102	30.7	2	
	12.11	82	31.5	2	After 2nd injection, 2 cc. pituitrin
8	11.05	164	19.0	1	Animal not eviscerated
	11.17	150	20.2	4	1 cc. pituitrin
	12.00	62	11.9	Occasional drop	
	12.11	80	15.0	2	2 cc. pituitrin

* Pressure earlier had reached 170 mm.

TABLE 2—*Concluded*

EXPERIMENT	TIME	BLOOD PRESSURE MM. HG. (CAROTID)	BLOOD FLOW THROUGH KIDNEY (CUBIC CENTIMETERS PER MINUTE)	URINE (DROPS PER MINUTE)	REMARKS
9	11.05	162	15.0	2	Animal eviscerated
	11.30	146	24.0	7	1 cc. pituitrin
	12.23	72	10.7	1	
	12.40	76	10.3	2	2 cc. pituitrin
10	12.47	98	19.4	0.5	Animal eviscerated
	1.07	124	38.4	5	1 cc. pituitrin injected
11	3.42	118	24.5	5	Animal eviscerated
	3.52	120	30.0	20	1 cc. pituitrin
12	3.43	84	20.0	3	Cat with tubular nephritis. Eviscerated.
	3.59	106	23.4	7	Injected 1 cc. pituitrin

TABLE 3

Influence of pituitary extract on the oxygen consumption of the kidney

EXPERIMENT	OXYGEN CONSUMPTION (CUBIC CENTIMETER PER GRAM PER MINUTE)		URINE FLOW	REMARKS
	Before injection	After injection		
1	0.118	0.109	Doubled	Animal not eviscerated
2	0.042	0.043	Slight increase	Animal not eviscerated
3	0.061	0.047	Increased fourfold	Animal eviscerated
4	0.068	0.066	More than doubled	Animal eviscerated
5	0.068	0.041	No increase in urine	Same animal as in 4; 50 min. later
6	0.047	0.049	Increased fourfold	Animal eviscerated
7	0.045	0.035	Very slight increase	Considerable loss of blood during operation
8	0.096	0.081	Definite increase	Animal eviscerated
9	0.045	0.037	No increase	
10	0.026	0.025	Doubled	Nephritis (tubular)

phate produced the decided increase in oxygen intake previously described by Barcroft and Straub.

Two special instances encountered during our experiments also suggest that the pituitary hormone and urea produce their effects through different mechanisms. In one case although the pituitary extract produced a definite increase in urine, subsequent injection of sodium sulphate failed to cause its usual marked effect. Later inspection showed

TABLE 4

Influence of urea and of sodium sulphate injections on the oxygen metabolism of the kidney

EXPERIMENT	SUBSTANCE INJECTED	OXYGEN CONSUMPTION (CUBIC CENTIMETER PER GRAM PER MINUTE)		URINE FLOW	REMARKS
		Before injection	After injection		
11	Urea, 2 gm. in 10 cc. saline	0.046	0.098	Good	Following pituitrin which caused no increase in oxygen intake
12	Sodium sulphate 20 cc. of 10 per cent solution	0.013	0.045	Marked	
13	Sodium sulphate, 10 cc. of 10 per cent solution	0.031	0.090	Marked	Preceding injection of pituitrin failed to cause increase in oxygen intake
14	Sodium sulphate, 5 cc. of 10 per cent solution	0.025	0.056	Slight	Later injection of pituitrin caused on increase of O_2 intake

that the kidneys were pathological and microscopical examination showed a marked tubular type of nephritis. It may be noted also that the oxygen consumption by this nephritic kidney was the lowest that we have measured. In another instance repeated injections of pituitrin failed to cause any urine flow. The subsequent injection of sodium sulphate was followed by a copious diuresis. Of course one can not put too much emphasis on such evidence yet the data fit in so well with the general picture that they at least warrant mention.

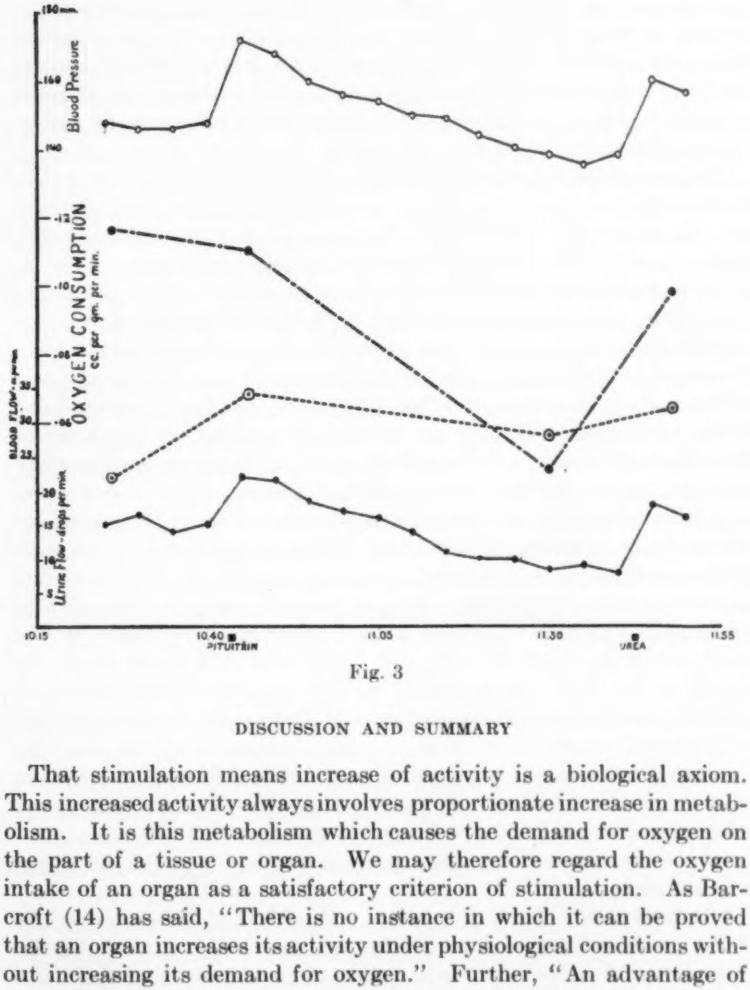

Fig. 3

DISCUSSION AND SUMMARY

That stimulation means increase of activity is a biological axiom. This increased activity always involves proportionate increase in metabolism. It is this metabolism which causes the demand for oxygen on the part of a tissue or organ. We may therefore regard the oxygen intake of an organ as a satisfactory criterion of stimulation. As Barcroft (14) has said, "There is no instance in which it can be proved that an organ increases its activity under physiological conditions without increasing its demand for oxygen." Further, "An advantage of the assurance that every increase in the activity of the cell means an instant call for oxygen lies in the fact that it furnishes a method of deciding whether in certain cases there is or is not increased activity on the part of the cell." This test for cell activity has been applied to the kidney by Barcroft and his coworkers. Thus the question of stimula-

tion has been answered in the affirmative for such diuretic substances as urea, caffein, sodium sulphate and phloridzin, and in the negative for the diuresis induced by Ringer's solution. We accordingly applied this test to the pituitary diuresis as it seemed to us that if we obtained a definite increase in the oxygen intake it could be regarded as a positive confirmation of Schäfer's conclusion.

Our results are in accord with Schäfer's in so far as they show that at times there is complete lack of parallel between the blood pressure and the urine flow. However the argument that this furnishes conclusive proof of the stimulation of the renal cells would seem to lose its force in the light of our findings relative to oxygen consumption and blood flow. It is difficult to explain the failure of the kidney to show any change in its metabolic activity on any basis of renal stimulation. The source of the energy must lie outside the kidney. While such possibilities as physical changes in the blood or changes in the permeability of the glomerular filter can not be entirely ignored, the relations of blood flow and urine flow are such that the effect may be satisfactorily explained as due entirely to the vascular change. Dilatation of renal vessels with more or less constriction elsewhere must be the causal accompaniment of increased blood flow. This by increasing the pressure in the capillaries of the glomerulus increases the rate at which the urine is formed. That the kidney vessels do show this specific dilatation in concentrations which cause constriction elsewhere is indicated by the work previously cited (7), (8), (9). Nor is this difference in the response of the renal vessels limited to their behavior toward pituitrin. Gottlieb and Magnus (15) state that digitalis has less constrictor effect on the renal vessels than on the mesenteric vessels and Bayliss (16) found that when the depressor nerve is stimulated the change in kidney volume and blood pressure are parallel while the volume of other internal organs increases. This he interprets as indicating a different organization of vasomotor mechanisms in the kidney as compared with other internal organs.

The outstanding effects of injections of extracts of the posterior lobe of the pituitary body are on involuntary muscle. What were earlier supposed to be evidences of stimulation of gland cells (e.g., the mammary) have more recently been explained as due to stimulation of the smooth muscle of the gland (17). We believe, on the basis of our results, that the action of the kidney is of the same general nature with the difference that in the case of the renal vessel the extracts caused inhibition (i.e., vasodilation) of the smooth muscle instead of stimulation. Our

results do not indicate that it is necessary to include a further effect on the cellular elements of the tubules. This vasodilation could hardly be passive as increased blood flow was found without accompanying rise of blood pressure. Several recent writers (18), (19) have reported that subcutaneous injections of pituitary extracts cause no change or may cause diminished production of urine during twenty-four hour periods. The question then of whether diuresis is produced, such as is usually obtained by intravenous injection of rather large doses, or whether there is diminished flow of urine is a question of whether the kidney vessels are dilated and an increased blood flow thereby maintained. It may possibly be found that when slower absorption occurs as from subcutaneous administration the renal dilatation may not be a prominent feature of the action; or when studied over long periods initial dilatation may be followed by a more prolonged constriction. Such results do not in any way disprove our findings.

The results of our experiments do not incline us toward the view that the posterior lobe extracts yield several hormones. The preparations that we used contained different amounts of the vasodilator substance but this is undoubtedly not specific but identical with that found in many organic extracts. The lack of parallel between the pressor effect on blood pressure and the effect on the kidney can well be explained on the basis of differing threshold of stimulation by the same active hormone.

CONCLUSIONS

1. The oxygen consumption by the kidney is not increased during the diuresis induced by pituitary extracts.
2. Using the oxygen consumption as the criterion, there is no evidence that pituitary extract stimulates the renal cells.
3. Throughout our experiments increased blood flow through the kidney was an invariable accompaniment of pituitary diuresis.
4. From the evidence at hand it seems possible to explain the diuretic action of pituitary extract entirely on the basis of the vascular changes and increased filtration pressure obtaining in the kidney.

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FURTHER STUDY ON THE EFFECT OF FOOD IN INCREASING OXIDATION

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Received for publication June 12, 1918

In a previous publication we (1) pointed out that the ingestion of the foodstuffs increased the catalase of the blood and hence of the tissues by stimulating the digestive glands, particularly the liver, to an increased output of this enzyme and accordingly suggested that the increase in oxidation after the taking of food, as observed by Lavoisier, was due to the increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide. It was also found that of the foodstuffs, meat was a more effective stimulant to the production of catalase than either fat or sugar and hence we suggested that the specific dynamic action of protein as set forth by Rubner (2) was due to this fact. The object of the present investigation was to determine the effect of the ingestion of the ordinary food materials, fruits and beverages on the production of catalase. The fatty foods used were bacon, lard, olive oil, glycerine and sodium palmitate; the protein foods, eggs, casein, peptone, beef extract and aminoids; the carbohydrates, starch, rice flour, corn meal, wheat flour, dextrine, honey, sucrose, maltose, lactose and dextrose; the fruits, oranges, bananas, lemons, rhubarb, apples, grape fruit; the beverages, chocolate, coffee, milk, cocoa and alcohol. After etherizing the animals and opening the abdominal wall, these substances were introduced in liquid form in about equal quantities into the stomach and intestine under pressure from a bottle through a rubber tube and an attached hypodermic needle. The quantity of the different substances used will be given in the description of the individual experiments. The catalase in 0.5 cc. of blood taken directly from the liver, the portal and jugular veins was determined before as well as at fixed intervals after the introduction of the different materials. The blood from the liver was collected from a superficial incision made in this organ. The catalase was determined by adding 0.5 cc. of blood to 50 cc. of hydrogen peroxide in a bottle at

approximately 22°C. and as the oxygen gas was liberated it was conducted through a rubber tube to an inverted burette, previously filled with water. After the volume of gas collected as described in ten minutes had been reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The material was shaken in a shaking machine at a fixed rate of one hundred and eighty double shakes per minute during the determinations. It will be noticed that none of the experiments lasted more than two hours owing to the fact that the animal with its abdominal wall open and having ether administered began as a rule to develop the condition of shock and on this account the catalase began to decrease.

In figure 1 under lard, bacon, olive oil, glycerine, sodium palmitate, trimethylene glycoll and ethyl alcohol are given curves constructed from data obtained from dogs before as well as at fixed intervals after the introduction of these materials into the alimentary tract. The lard was introduced in the form of an emulsion which was made by shaking 500 grams of lard heated to 40°C. with 200 cc. of a 1 per cent sodium carbonate solution. Five hundred grams of finely ground bacon were tried out and introduced into the alimentary tract. Four hundred cubic centimeters of glycerine were shaken with 200 cc. of water and introduced as such. The sodium palmitate was prepared by adding sodium hydroxid to 350 grams of palmitic acid. Five hundred cubic centimeters of 50 per cent trimethylene glycoll and 500 cc. of 45 per cent ethyl alcohol were used. All the substances were warmed to 38 C. previous to being introduced into the alimentary tract of the dogs. The figures along the ordinate in chart 1 as well as in all the charts in this paper represent amounts of catalase measured in cubic centimeters of oxygen liberated by 0.5 cc. of blood from 50 cc. of hydrogen peroxide in ten minutes, and the figures along the abscissa, time in minutes. The continuous line curves in all the charts of the paper were constructed from data obtained from blood taken from the liver, the discontinuous line curves from the blood of the portal vein, and the dotted line curves from the blood of the jugular. It will be seen in figure 1 that previous to the introduction of the lard, 0.5 cc. of the blood of the liver liberated 74 cc. of oxygen from hydrogen peroxide while 0.5 cc. of blood from the portal and jugular veins liberated 70 and 69 cc. of oxygen respectively. After the introduction of the lard the catalase in the blood of the liver, portal and jugular veins was increased as is indicated by the increase in the amount of oxygen liberated from

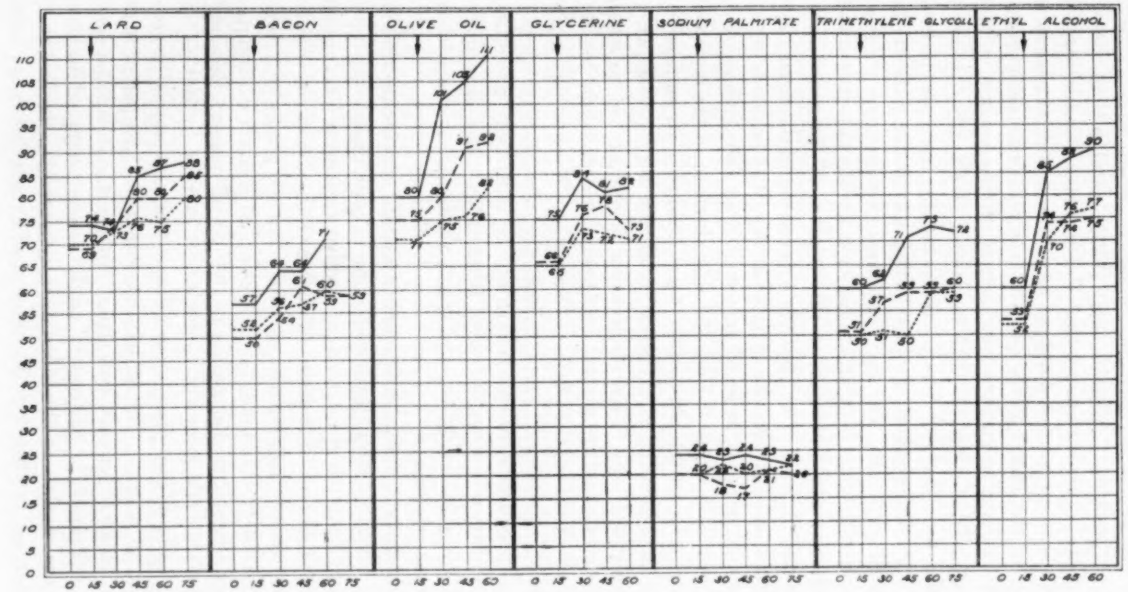

Fig. 1. Curves showing effect of the introduction of lard, bacon, olive oil, glycerine, sodium palmitate, trimethylene glycoll and ethyl alcohol into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of portal vein; - - - - - curves show amount of catalase in the blood of jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

hydrogen peroxide. The catalase of the portal blood was increased more rapidly particularly during the first thirty minutes than that of the jugular. This is taken to mean that the lard was stimulating the alimentary glands to an increased output of catalase while the increase in the catalase of the blood of the jugular was due principally to the stimulation of the liver to an increased output of this enzyme. That the liver is normally putting out catalase continuously into the blood is shown by the fact that the blood taken directly from the liver is always richer in catalase than the blood from any other part of the body by 15 to 20 per cent. The fact, as shown in a previous publication, that the introduction of a substance such as ethyl alcohol which normally produces a great increase in the catalase of the blood, produced a very small increase after the liver was cut out of the circulation by an Eck fistula and ligating the hepatic artery, was taken to mean that the liver is the organ principally responsible for the production of catalase. It may also be seen that the introduction of the bacon as well as the olive oil into the alimentary tract produced an increase in catalase. The introduction of glycerine produced an increase while sodium palmitate did not. This observation is interpreted to mean that the glycerine radical in the fat molecule is responsible for the stimulating effect of fat on the output of catalase. By comparing the stimulating effect of glycerine, trimethylene glycoll and ethyl alcohol to an increased production of catalase it will be seen that glycerine, a trihydroxy alcohol, was least effective, trimethylene glycoll, a dihydroxy alcohol was more effective, and ethyl alcohol, a monohydroxy alcohol, was most effective.

In figure 2 the curves were constructed from data obtained before as well as at fixed intervals after the introduction of eggs, casein, peptone, beef extract and biuret-free aminoids. Twelve eggs were beaten up and strained through two thicknesses of cheese cloth and introduced in this form. The casein was prepared by dissolving 400 grams of a commercial preparation of this material in 200 cc. of sodium carbonate; the peptone by dissolving 350 grams in 300 cc. of water; the beef extract by dissolving 300 grams of Liebig's beef extract in 200 cc. of water, and the aminoids by dissolving a biuret-free commercial preparation in 200 cc. of water. The aminoids contained 11.98 per cent of nitrogen and of this 8.9 per cent was amino nitrogen. It will be seen that the introduction of the egg as well as the casein produced little or no increase in the catalase of the blood as is indicated by the fact that there was no increase produced in the amount of oxygen liberated from

hydrogen peroxide. The peptone and beef extract produced an increase but not so marked as did the aminoids. It is assumed that the egg and casein did not produce an increase because the length of the experiment was not sufficient to permit the digestion and absorption of these substances, while the peptone, beef extract and aminoids, being

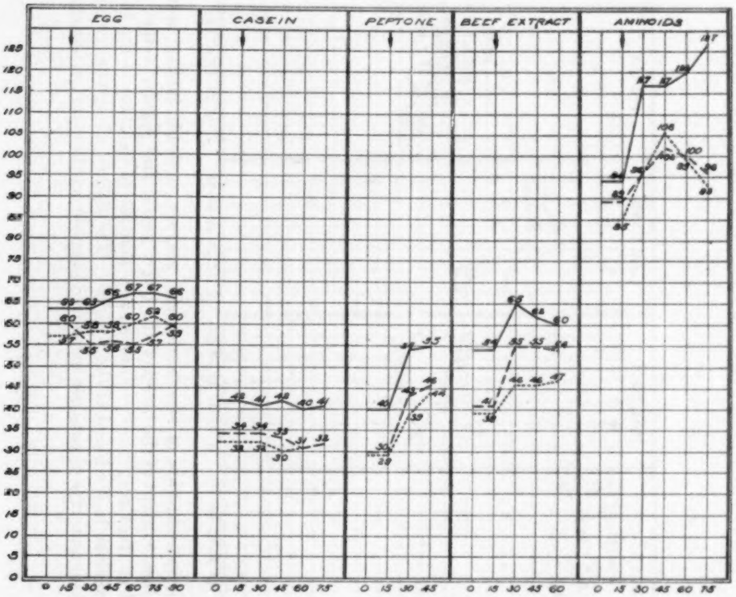

Fig. 2. Curves showing effect of the introduction of egg, casein, peptone, beef extract and aminoids into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

immediately absorbed, stimulated the digestive glands, particularly the liver, and thus produced an increase in catalase. According to Pawlow beef extract functions in stimulating the gastric glands to an increased output of gastric juice. The preceding observation shows that beef extract also stimulates the liver to an increased output of catalase. The fact that the aminoids being composed mostly of amino acids

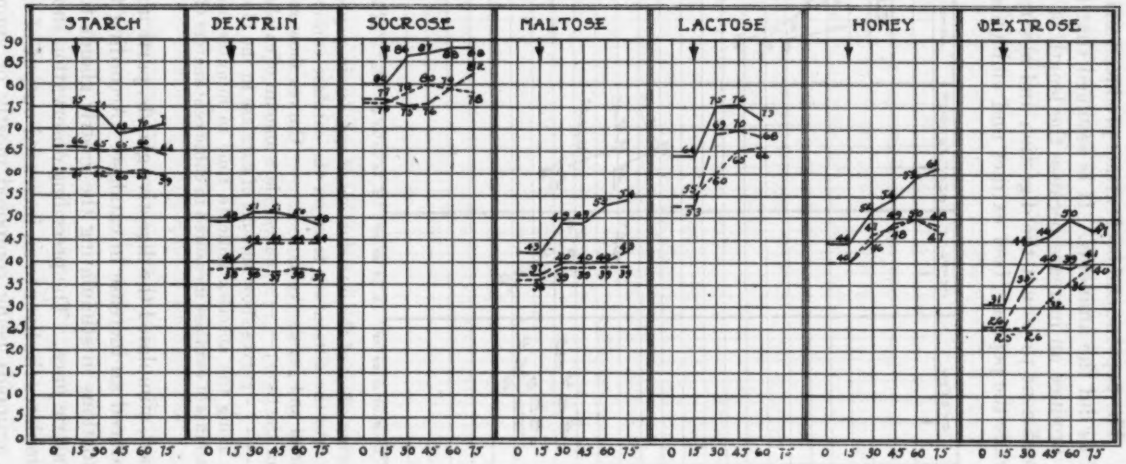

Fig. 3. Curves showing effect of the introduction of starch, dextrin, sucrose, maltose, lactose, honey and dextrose into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

stimulated the digestive glands to the greatest extent suggests that the amino acids are the substances responsible for the great stimulating effect of meat on the production of catalase.

In figure 3 under starch, dextrine, sucrose, maltose, lactose, honey and dextrose are given curves constructed from data obtained from dogs after the introduction of these materials into the alimentary tract. The starch was introduced in the form of a paste which was made by adding 200 cc. of water to 400 grams of corn starch. Four hundred grams of dextrine as well as each of the sugars used were dissolved separately in 200 cc. of water. It will be seen that the introduction of the starch as well as the dextrine produced little or no increase in the catalase of the blood while the sugars produced an increase, lactose and dextrose producing the greatest increase. It is assumed that the starch and dextrine did not produce an increase because the length of the experiment was not sufficient to permit the digestion and absorption of these substances. The dextrose produced a more rapid and extensive increase in catalase than did sucrose or maltose due presumably to the greater rapidity with which it is absorbed. Why lactose, a disaccharide should have produced a more rapid and extensive increase than the other two disaccharides, we are unable to say. However the purposefulness of this becomes evident when it is considered that lactose is the sugar on which suckling animals live. The stimulating effect of honey was due presumably to the simple sugars constituting the bulk of this substance.

In figure 4 are shown curves constructed from data obtained before as well as at fixed intervals after the introduction of rice flour, corn meal, wheat flour, dextrine and dextrose. These substances were prepared for injection by adding 200 cc. of water to 400 grams of each of the materials and warming to 38°C. It will be seen that the introduction of none of these substances except dextrose produced an increase in catalase, due presumably to the length of time of the experiment being too short to permit the digestion and absorption of the materials.

In figure 5 under oranges, oranges (very ripe), bananas, lemons, rhubarb, apples and grape fruit are given curves constructed from data obtained from dogs previous to and after the introduction of these materials into the alimentary tract. Two dozen oranges were peeled and ground to a very fine consistency. Half of this material was used immediately while the other half was permitted to stand in a thermostat at 38°C. for twenty-four hours. The material that had stood in the thermostat is referred to in the chart as oranges (very ripe). Twelve

bananas were peeled, macerated and also placed in a thermostat for twenty-four hours at 38°C. The pulp and the juice of twelve lemons and of four large grape fruit were ground up separately and strained through a cheese cloth. Similarly 500 grams of rhubarb and of apples were prepared. It may be seen that the introduction of the oranges produced no increase in catalase while the introduction of oranges (very ripe) as well as the bananas which had undergone the ripening process produced an increase in the catalase of the blood. The stimu-

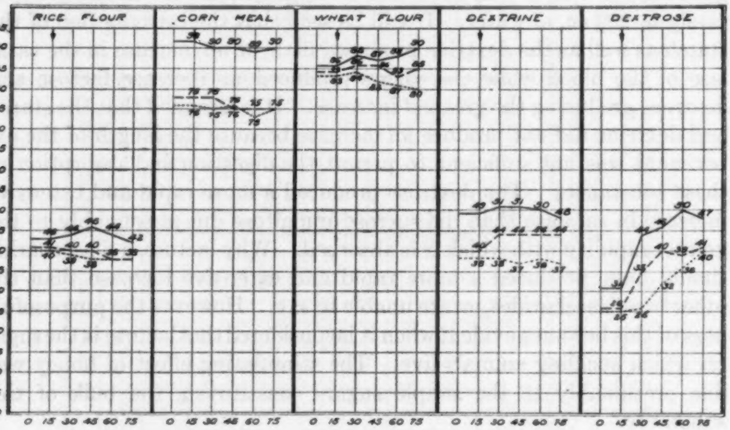

Fig. 4. Curves showing effect of the introduction of rice flour, corn meal, wheat flour, dextrine and dextrose into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

lating effect of the fruit which had undergone the ripening process is attributed to the large amount of sugar and possibly alcohol in this material. The introduction of the macerated lemons and apples produced very little if any increase while the rhubarb and grape fruit produced a very small increase in catalase.

The chocolate and cocoa used in the experiments in figure 6 were prepared by dissolving 200 grams of Baker's chocolate and 200 grams of cocoa in 300 cc. of water. The coffee was prepared by adding 50 grams

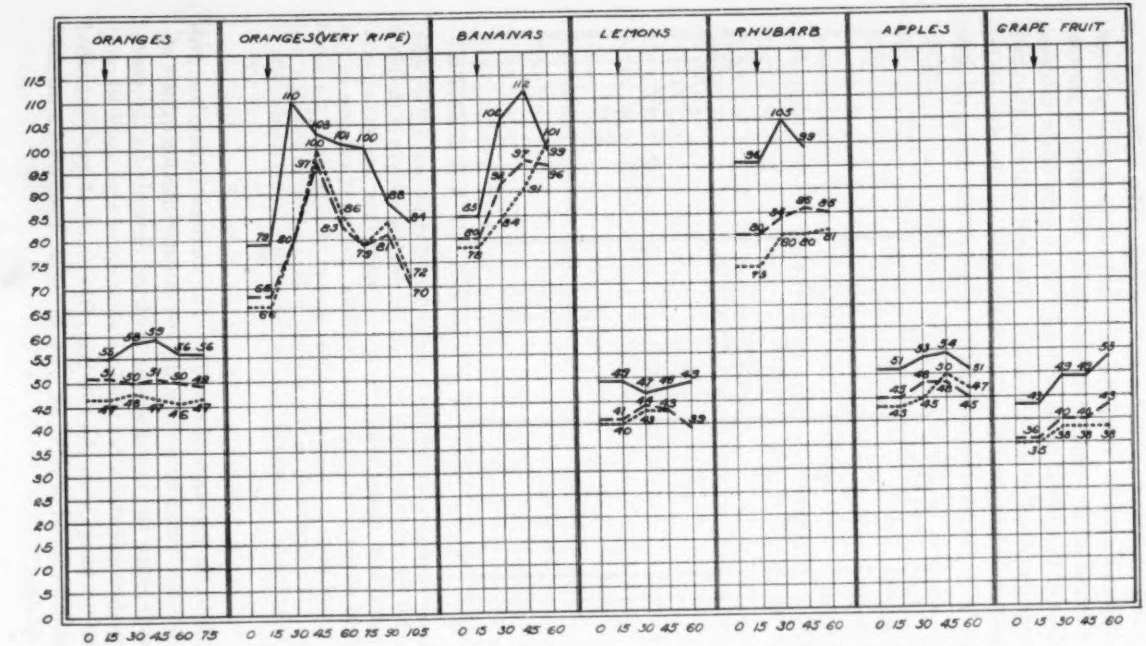

Fig. 5. Curves showing effect of the introduction of oranges, oranges (very ripe), bananas, lemons, rhubarb, apples and grape fruit into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

of this material to 400 cc. of water and boiling. The milk used was 500 cc. of fresh cow's milk. It will be seen that the chocolate produced a marked increase in the catalase of the blood, the coffee an increase in the catalase of the portal blood and the milk a very slight increase, while the cocoa produced no increase at all. The only difference between the cocoa and chocolate used was that the former contained much less fat than the latter, hence the stimulating effect of the chocolate must

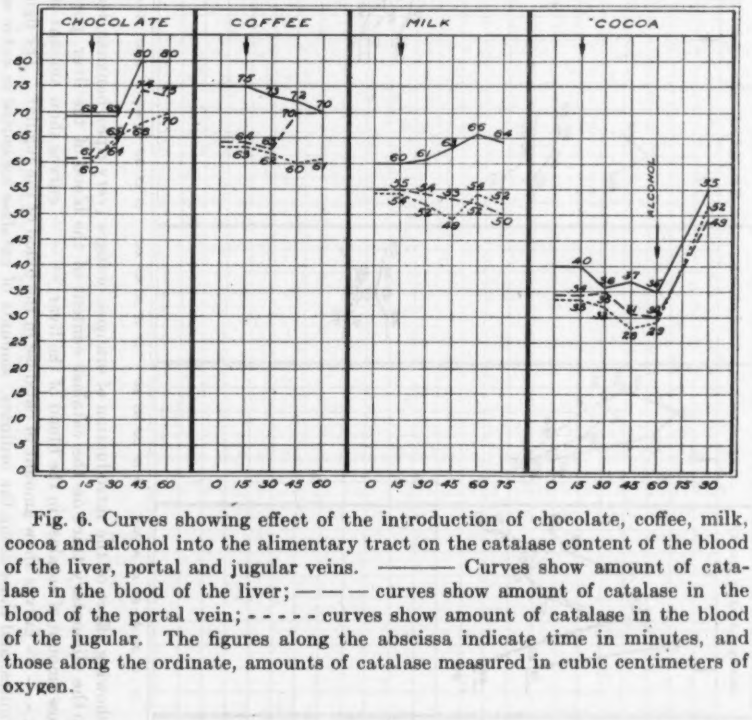

Fig. 6. Curves showing effect of the introduction of chocolate, coffee, milk, cocoa and alcohol into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

have been due to the presence of the fat. The increase in catalase produced by the coffee must have been due to the stimulation of the alimentary glands since the increase was confined entirely to the portal blood. Forty-five minutes after the introduction of the cocoa when it had produced no increase in the catalase, 500 cc. of 45 per cent ethyl alcohol were introduced which, as may be seen, produced a marked increase in catalase.

Lavoisier showed that the ingestion of food increased oxidation in the body. Rubner showed that of the foodstuffs meat ingestion increased oxidation most, fat next and sugar least. Magnus-Levy (3) showed that the ingestion of 320 grams of fat bacon increased metabolism 19 per cent and the ingestion of 210 grams of butter increased it 14 per cent. Johansson, Billström and Heijl (4) showed that ingestion of the simple sugars increased metabolism. Means, Aub and DuBois (5) showed that the administration of large doses of caffeine to normal individuals produced an increase in the basal metabolism 10 to 30 per cent. Lusk (6) showed that the ingestion of olive oil as well as amino acids increased metabolism and accordingly suggested that the stimulating effect of protein to increased heat production is due to the influx of amino acids or organic acids resulting from the deamination of the amino acids. Benedict (7) claims that the ingestion of carbohydrates increases oxidation by the formation of acid intermediary products which stimulate metabolism. Voit (8) believed that the presence of increased quantities of food materials augmented the inherent power of the cells to metabolize, while Rubner contended that the increased heat production which follows the taking of food was due to heat developed from intermediary reactions and oxidations which were in no way involved in the life processes of the cells. It may be seen in this paper that all of the substances which the different investigators found to increase oxidation also increased the catalase of the blood and hence of the tissues by stimulating the digestive glands, particularly the liver, to an increased output of this enzyme. The increase in oxidation after the taking of food may be due to the increase in catalase. The fact that when oxidation is increased in the body, as for example, by increasing the amount of work, by thyroid feeding, by fighting, in the excitement stage of anaesthesia, there occurs a corresponding increase in catalase and that when oxidation is decreased, as for example, by decreasing the amount of work, by starvation, by phosphorus poisoning, in deep narcosis, in "surgical shock," or rendered defective as in pancreatic diabetes, there results a decrease in catalase, would seem to offer further evidence in this direction. It may be mentioned in this connection that we have found that when heat production was raised to higher levels by the ingestion of larger quantities of meat as observed by Rubner (9), there occurred a corresponding increase in catalase. The catalase content of the blood of a poorly fed dog was also found to be increased by feeding the animal well with the ordinary food materials. It was also found that there was an increase in the catalase of

the blood during moderate exercise while there was a decrease during very severe exercise and fatigue. During the period of recovery from fatigue the catalase rose to the normal amount. If catalase is the enzyme principally responsible for oxidation in the body, it may be that the decrease in catalase with resulting decrease in oxidation is one of the factors involved in the production of fatigue and the increase a factor in the recovery from fatigue. Of the four or five hundred dogs that have been used in connection with our work on catalase during the past three years, we have observed that the catalase of the blood of lively, energetic dogs is almost invariably high while that of less energetic dogs is lower. As a rule one can pick out the dogs of high catalase on the basis of liveliness. The amount of oxygen liberated from hydrogen peroxide by the blood of sluggish dogs rarely exceeds 30 or 40 cc. and may be as low as 10 cc. in certain very sluggish dogs, while the amount liberated by the blood of lively dogs rarely falls below 70 or 80 cc. and may be as high as 175 cc. in certain very active animals.

SUMMARY

The increase in oxidation following the ingestion of food is attributed to the increase in catalase produced by the stimulation of the digestive glands, particularly the liver, to an increased output of this enzyme. The glycerine radical of the fat molecule is responsible for the stimulating effect of the fats; the end products of protein digestion, presumably the amino acids, for the stimulating effect of meat; and the simple sugars for the stimulating effect of the starchy foods.

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MATERIAL LOST IN MENSTRUATION OF HEALTHY WOMEN

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Received for publication June 19, 1918

In connection with metabolism experiments recently reported (1), (2), it has been possible to secure data upon the losses of nitrogen, phosphorus, calcium and iron in the menstrual flow. The results may be of interest to readers of this Journal in connection with the studies on the "periodic cardiovascular and temperature variations in women" published by King (3).

King's results show slight but irregular changes in temperature, pulse and blood pressure previous to and during the menstrual period although the changes throughout the month are often as marked as those at the menstrual period. She feels however that there is considerable consistency in the variation at this period so that her conclusion is that there is a slight but periodic movement in the physiological processes of women.

In the experiments referred to in this paper the subjects were healthy women who were working in the laboratory from ten to twelve hours a day. The main object of the experiments and the details with regard to diet have been carefully described in the papers referred to above. In general the object was to ascertain whether the calcium and phosphorus requirements of women differed materially from those of men. The diet of each subject was uniform from day to day and the daily intake and output for nitrogen, calcium and phosphorus were determined by analysis. The menstrual flow was collected on absorbent cotton, extracted frequently in tepid distilled water and the separate extractions preserved as one sample. The volume was kept as small as possible—usually below 2000 cc.—and the sample was preserved with thymol. At the end of the period samples of the solution and the used cotton were analyzed for nitrogen, calcium and phosphorus. Samples of the unused cotton were also analyzed and the amounts of nitrogen, calcium and phosphorus found in this material deducted from

the amount in the used cotton. The results are shown in the following table:

TABLE I

Nitrogen, phosphorus and calcium output in the menstrual flow. These results compared with the average total daily output for the same period

	WEIGHT	LENGTH OF MENSTRUAL PERIOD	TOTAL AMOUNT IN THE MENSTRUAL FLOW			AVERAGE DAILY OUTPUT IN MENSTRUAL FLOW			AVERAGE TOTAL DAILY OUTPUT THROUGH URINE AND FECES DURING THE MENSTRUAL PERIOD		
			Nitrogen	Phosphorus	Calcium	Nitrogen	Phosphorus	Calcium	Nitrogen	Phosphorus	Calcium
	kgm.	*days*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Subject K:											
Exp. 1......	52	4	1.92	0.052	0.016	0.48	0.013	0.004	8.44	0.69	0.29
Exp. 2......	50	5	1.54	0.030	0.020	0.31	0.006	0.004	7.04	0.71	0.41
Subject M:											
Exp. 1......	57	4	3.24	0.052	0.0056	0.81	0.013	0.0014	10.86	0.74	0.22
Exp. 2......	60	4	3.36	0.060		0.84	0.015		8.09	0.72	
Subject N:											
Exp. 1......	54	5	3.15	0.045	0.008	0.63	0.009	0.002	10.64	0.81	0.35
Exp. 2......	55	5	1.85	0.030		0.37	0.006		6.66	0.71	

The total amount of calcium and phosphorus lost through the menstrual flow as shown by the above table is small, and while the handling of the sample is necessarily crude, the figures are at least suggestive that there is not a large loss of either of these elements at the monthly period. The nitrogen loss is larger than that of either the calcium or the phosphorus but when viewed in the light of the total output for the month is not very significant.

In experiment 2 for subject K and in experiment 1 for each of subjects M and N iron was determined on the sample with 34, 38 and 42 mgm. of iron respectively for the menstrual period of each of these three experiments. When viewed in the light of the standard for daily requirement of iron (from 10 to 15 mgms.), the loss of iron at this period is probably an important factor and should be considered by women in choosing their daily diet. The loss of iron then seemed to be more significant than any of the other elements investigated.

A study of the daily output of these six experiments (1), (2) seems to give very little basis for discussing nitrogen retention either previous

to or during the menstrual period. In experiment 1, for subject K there was a noticeable retention of both nitrogen and calcium on the first day of the period. The output of nitrogen for the day preceding the period, for the four days of the period and for the day following the period is as follows: 8.84, 7.00, 9.49, 8.47, 8.80 and 8.08 grams respectively. On only two other days during the month did the nitrogen output fall below 8.00 grams, the lowest of these being 7.48 grams for the second day of the experiment. The average daily output for the month was 8.72 grams. This is the only experiment which shows a definite nitrogen retention at the beginning of the menstrual period.

In two of the six experiments the menstrual period was so near the beginning of the experiment that the output may have been influenced by the previous diet. In the three remaining experiments the nitrogen output for the first day of the period or of the preceding day was only slightly lower than for the few days previous, making a difference so small as to come within the range of experimental error and since there were several other isolated days throughout the month when the output was as low or lower than the output for this day it would be impossible to say definitely that there was a regular retention at this period. Experiments covering more than one menstrual period with several subjects would be required before definite conclusions could be drawn.

These results show as did those of King that there are as great variations during the intermenstrual periods as at the period itself and that the tendency to a variation at the menstrual period is slight

To compensate however for the diminished urinary nitrogen excretion at the beginning of the menstrual period as previously observed (4), (5), there is the extra output of nitrogen in the menstrual flow. It contains from 1.5 to 3.0 grams of nitrogen for the period, or an average of 0.3 to 0.6 gram per day during the period, which is equivalent to 0.05 to 0.1 gram per day for the month.

Of phosphorus and calcium the amounts lost through the menstrual flow were only about 0.03 to 0.06 gram and 0.01 to 0.02 gram respectively for the period—quantities which are smaller than the chance variations in the output from day to day.

The data therefore show that there is no pronounced periodicity in the output of phosphorus and calcium and that the amounts of these elements lost in menstruation are not sufficient to make the nutritive requirements of women for these elements materially different from those of men of the same weight. The figures for iron however may indicate a more significant difference.

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MUSCULAR STRENGTH AND MUSCULAR SYMMETRY IN HUMAN BEINGS

II. In Adult Males

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Received for publication June 19, 1918

Determinations of muscular strength in human beings by means of dynamometers have been made in large numbers by numerous investigators in connection with various problems (1). Among the problems so approached may be mentioned studies of fatigue (2) and of relationship between physical strength and intelligence (3). The favorite instrument in these studies has been the grip dynamometer, either the old form of Collin or the improved type designed by Smedley (1). In spite of the objections that have been urged against the grip dynamometer as an instrument of research, much valuable information has been obtained with its aid (1). It presents, however, the undoubted shortcoming of making use of one of the most complex musculatures of the body and a musculature, moreover, which receives much special training. The question may properly be raised whether the strength of the grip truly represents general bodily strength or whether this particular musculature may not reflect special conditions to an exceptional degree. A test of strength, to be quite satisfactory, should afford information of the strength of the body as a whole. Ideally it should include all the important muscle-groups; practically a strength test is valid if proof can be furnished that it constitutes a fair sample of the entire strength.

The system of muscle-testing in course of presentation in the series of papers of which this is the second (4) differs from the systems previously proposed in the fundamental fact that it measures "breaking strength" of muscles, namely, the force required to overcome maximal resistance rather than the utmost positive effort, as in other methods. Which system is better can be determined finally only by continued experience and comparison. Certain advantageous features of the

present system may be mentioned. As a routine feature of the test the subject is required to develop resistance at the command "hold back" uttered sharply. We find that under this stimulus the maximum effort is elicited, almost as a reflex. Only by deliberate planning on the

TABLE 1

Average percentage distribution of strength among the muscles, adult males. For comparison the distribution for children of 13 to 18 years is given also

MUSCLE-GROUP	ADULT MALES	13 TO 18 YEARS
Feet		
Plantar flexion	10.00	9.30
Dorsal flexion	2.85	3.20
Inversion	1.90	2.10
Eversion	1.80	2.00
Thighs		
Adduction	1.50	1.55
Abduction	1.40	1.50
Extension	3.70	3.00
Flexion	3.20	3.10
Knees		
Extension	3.30	3.30
Flexion	1.75	1.70
Shoulders		
Pectoralis	2.35	2.10
Latissimus dorsi	1.70	1.45
Anterior deltoid	2.10	2.00
Posterior deltoid	1.40	1.50
Forearms		
Extension	1.50	1.60
Flexion	2.25	2.50
Wrists		
Extension	1.05	1.35
Flexion	1.35	1.90
Fingers		
Extension	0.65	0.70
Flexion	2.95	2.75
Thumbs		
Adduction	1.30	1.40

part of the subject will less than his best effort be put forth. Thus an element of reliability is introduced, which is of the highest importance. As a matter of fact the deliberate attempt to make an inferior showing is instantly revealed in the changed character of the response. Since the spring balance with which the strength is determined is held in the

hands of the operator, the line of pull can be rectified in accordance with the position of the subject. The maintenance of correct lines of pull is of course essential to the validity of any system of strength-testing. The promptness with which the correct position can be taken is a feature of this method which becomes valuable as a saver of time when large series of tests are being carried on.

The muscle-test. The system of muscle-testing as originally developed is described elsewhere (5). The list of muscles included in the complete test is set down in table 1. For the series of tests on adults on which this paper is based certain muscle-groups were regularly omitted. These were *a*, the plantar flexors, which are so strong in adults as to make their testing a matter of inconvenience, since a lever has to be arranged for multiplying the force of the spring-balance pull; *b*, the finger extensors, which were omitted because their short leverage requires the substitution of a special narrow sling for the full-width sling used with the other muscle-groups, a time consuming substitution, and in this particular series the saving of time was an important consideration. Tests of finger flexors were made on only part of the subjects. Here the short leverage, together with the great strength, marks the group as less satisfactory than most of the others. Tests of the wrist flexors were omitted from part of the early subjects on account of a fear, found later to be unjustified, that testing them might give discomfort.

Subjects. The adults tested were students or instructors in this University. For assistance in obtaining the coöperation of these individuals we are indebted to the Department of Military Training of Stanford University, for whose courtesy we wish to express our thanks. In addition to the fifty adult males on whom the report is primarily based, we had access to the records of an equal number of adult females tested by Doctor Mosher and one of us (6). There were also available the records of fifteen adults from the series of infantile paralysis cases reported in a former paper (4), and more than a hundred "short" tests (p. 34) made from time to time as opportunity offered. In many cases duplicate records were made at least eight hours apart; about one-fourth of the subjects were tested five or more times.

Object of the experiments. Our object in these experiments was in part the establishing for adults of the constants previously worked out for children (4). In addition to this, however, we had in mind the possibility that this system of testing might offer certain advantages for the routine determination of strength provided it could be shown to be scientifically reliable and practical in application.

The complete test, as outlined in table 1, can scarcely be carried through in less than a half-hour. Clearly, for practical purposes, some abbreviation is highly desirable. A feasible method of abbreviation would be to make actual determinations of strength of a few muscle-

TABLE 2

Coefficients of correlation, individual muscle-groups to total strength, 56 adults

MUSCLE-GROUP	COEFFICIENT OF CORRELATION	
	Right	Left
Feet		
Plantar flexion		
Dorsal flexion	0.86	0.83
Inversion	0.68	0.66
Eversion	0.7[illegible]	0.66
Thighs		
Adduction	0.815	0.81
Abduction	0.885	0.89
Extension	0.93	0.87
Flexion	0.89	0.875
Knees		
Extension	0.83	0.865
Flexion	0.87	0.93
Shoulders		
Pectoralis	0.92	0.87
Latissimus dorsi	0.73	0.76
Anterior deltoid	0.77	0.79
Posterior deltoid	0.59	0.64
Forearms		
Extension	0.825	0.82
Flexion	0.89	0.91
Wrists		
Extension	0.61	0.585
Flexion	0.60	0.64
Fingers		
Extension		
Flexion	0.47	0.50
Thumbs		
Adduction	0.80	0.78

groups only and to compute from these the total strength. That such a method is valid is suggested in a former paper (Martin: loc. cit., p. 72). Our task here is to verify the validity of this idea and to examine the different muscle-groups individually to see which should be included in the abbreviated test. Since the first criterion of an abbreviated test

must be its reliability, namely, the showing that it represents fairly the total strength, the muscles selected for inclusion should be those that show the highest correlation of individual strength with the total strength, provided the muscles are found to differ in this regard.

For comparing the strength of individual muscles with the total strength of the body we used a series of fifty-six cases on which complete tests (except of plantar flexors and finger extensors, as noted above) had been made. Records of wrist flexion and of finger flexion were missing also from a part of the cases. These cases were tabulated and the Pearson coefficient of correlation of each muscle with the total strength calculated. The results are set down in table 2. Inspection of this table shows that the muscles vary among themselves in the extent to which their individual strength tends to bear a fixed relation to the total strength. There are ten pairs in which the correlation of each muscle of the pair with the total strength is above 0.80, with a probable error of not to exceed ±0.032. Evidently a short test will be most reliable if selected from among these ten pairs of muscle-groups. Incidentally it may be noted that the lowest coefficients of correlation in the entire series were given by the finger flexors, which are the muscles used in tests of strength with grip dynamometers. It is probable that these coefficients are lower than would be given by tests taken with perfected instruments of the Smedley type, but the fact which seems to be generally true, to judge from table 2, that the muscle-groups with short leverage correlate less closely than do those with long leverage, indicates that the original selection of the grip as a criterion of bodily strength was perhaps unfortunate.

In making selection for a short test from among the ten pairs of muscle-groups whose correlations with total strength are satisfactorily high, the determining criterion would seem to be altogether that of suitableness for the practical procedure of the test. The application of this criterion narrows at once the range of selection. Thus hip extensors, hip flexors and knee extensors, although correlating well with total strength, are undesirable from the practical standpoint because they are very strong muscles, requiring often a tension of two hundred pounds or more to overcome their resistance, and the great labor involved in developing this high tension repeatedly is likely to prove too exhausting to the giver of the tests. The knee flexors are among the most satisfactory muscles tested on children, but with adults they are likely to cramp when contracted forcibly, a fact which constitutes a valid objection to their use in a routine test. There remain in the

available list six pairs of muscle-groups, three on the legs: dorsiflexors of the foot, adductors of the thigh, abductors of the thigh; and three on the arms: pectorals, extensors of the forearm, flexors of the forearm. Minor considerations suggest the elimination from the short test of the dorsiflexors of the foot and the extensors of the forearm: the first because removal of the shoe would be necessary, the second because testing of the forearm extensors involves a degree of care in placing the parts in position which militates against speed in carrying out the test. With the idea that the test might be found available in industrial studies we have striven to reduce the time required to the least possible figure. The four pairs of muscle-groups now included in the list are the adductors and abductors of the thigh, the pectorals and the flexors of the forearm. Without any real justification but merely from a feeling that the muscles which move the hand ought to have representation in the short test, we added originally to this list the flexors of the wrist. Additional experience has convinced us, however, that the added information thus obtained is not of sufficient importance to justify the additional time consumed in testing the wrist muscles; we omit them, therefore, from our standard short test as here proposed.

For the sake of completeness, and also in order that simple directions may be readily available, the technique of the short test is here outlined.

Apparatus required. An ordinary flat-face spring-balance with a scale capacity of 200 pounds by 2 pounds, equipped with a self-registering index. (There are scales on the market with self-registering indices but these are heavier than desirable. We have found it satisfactory to fit up an ordinary scale in our own shop with a simple device.) A stout wood handle is attached by a swivel to the upper end of the scale, and a loop of stout leather 1¼ inch wide and 30 inches in circumference is attached by another swivel to the lower end of the balance.

A stout table 6½ feet long and 2½ feet wide, with a cleat secured firmly across one end. A cushion on which the subject's head may rest should be provided with this table,

An upright post 4 inches square and at least 6½ feet high, so placed that it is surrounded on at least three sides by ample space. Some form of hand-hold is provided by which the subject may steady himself as he leans against the post. (A knotted rope tied to a convenient ring near by answers well for this hand-hold.)

Procedure: General instructions. The individual to be tested is referred to as the subject. The persons giving the test are: first, the adjustor; second, the operator.

The duties of the adjustor are to place the loop in the assigned position about the arm or leg, support it there with one hand and, if necessary, the arm or leg of the subject with the other. He gives the command "hold back" to mark the beginning of the pull, and "stop" to mark the end.

The operator has the handle of the balance in his right hand and the body of the balance in his left.

After the loop is adjusted the adjustor gives the command "hold back." At this command the subject contracts with all his power the muscle-group being tested and simultaneously the operator pulls upon the spring-balance. Tension must be developed as rapidly as possible *without jerking*, and must be increased until the resistance of the subject is actually overcome. At the command "stop" the pull is discontinued immediately. The scale is read at once and the reading recorded by the assisting clerk. The sliding indicator of the scale must always be returned to the zero position immediately.

Tests are taken with the subject fully dressed.

Muscle-groups that are reported by the subject to be sore are not tested.

Calculation of total strength. The sum of the strengths shown by the individual muscles included in the short test constitutes 15 per cent of the entire strength as found by the complete test (see table 1). To calculate the entire strength, therefore, the sum of these determined strengths must be multiplied by the reciprocal of 0.15, which is 6.67. The product thus obtained is the figure for the strength of the subject. If for any reason any muscle-group was omitted from the test, assume the strength of the omitted muscle to be the same as that of the corresponding muscle on the other side.

Detailed technique of the tests. a. Pectorals. The subject stands at attention with the middle of his back pressed firmly against the upright post and the hand of the arm not being tested grasping the hand-hold. The arm to be tested is allowed to be limp in the hands of the adjustor until the command "hold back," with which command the pectoral muscles are contracted as strongly as possible. The adjustor stands directly in front of the subject, facing him; places the loop of the balance about the arm to be tested, just above the elbow; with one hand he holds the loop in position and grasps lightly the subject's hand or wrist with his other hand. Keeping the subject's arm straight, the adjustor draws it across the subject's body as far as possible, keeping it as close to the body as can be done and still give clearance for the loop. At the command "hold back" the subject's effort is to hold the arm from being drawn backward and downward from this position. The operator, standing at the subject's side, holds the balance in a line downward and backward from the subject's elbow in such a position that the arm as drawn back will just clear the subject's body. At the command "hold back" the operator develops sufficient tension to draw the arm down to the side of the body. The command "stop" must be given and the pulling discontinued before the arm has been drawn beyond the vertical line.

b. Forearm flexors. The subject lies on his back on the table with his heels pressed firmly against the cleat. The adjustor stands at the subject's left for both flexors. His right hand holds the subject's elbow to the table; his left hand brings the subject's forearm into a position of flexion about 15 degrees toward the shoulder from the vertical, and adjusts the loop about the wrist so that its upper edge is at the crease in the skin at the base of the hand. The operator stands at the foot of the table; he develops tension at the word of command. The command "stop" should be given when the forearm reaches the vertical.

c. Thigh adductors. Position of the subject same as in the above test except that he presses against the cleat only with the foot of the leg that is not to be

tested. He may steady himself by grasping the edges of the table. The adjustor stands at the foot of the table; with one hand he places the loop in the hollow just above the malleolus (an equally correct index is to have the loop just clear of the top of an ordinary man's shoe); he seizes the subject's heel with the other hand; lifts the leg until the heel is just high enough to clear the other toe, and then draws the leg into extreme adduction. The toe of the leg to be tested must be kept vertical. The operator stands at the side of the table and develops tension at the word of command. The command "stop" should be given as soon as the leg is drawn into line with the axis of the body.

d. Thigh abductors. The position of the subject and of the adjustor is the same as in the above test. The loop is placed as for the adductors except that the direction of pull is opposite. The leg to be tested is drawn out 15 degrees beyond the line of the body; the effort of the subject at the command "hold back" is to prevent the operator from drawing the leg into line with the body. The command "stop" is given just as the leg reaches the midline.

The most convenient order for the tests is as follows:

Right pectoral
Left pectoral
Right forearm flexor
Left forearm flexor
Right thigh adductor
Left thigh abductor
Right thigh abductor
Left thigh adductor

Although the satisfactory giving of the test requires careful training and considerable practice on the part of operator and adjustor, the demands upon the subject are not great. We have made successful tests upon subjects with a very limited knowledge of English and only ordinary intelligence. Much time can be saved by letting subjects not yet tested see the test carried out on others. In this way they learn what is expected of them and carry out their part promptly when their turn comes. A few seconds over one minute is usually enough time for carrying out a short test.

Certain relationships which can readily be carried in mind are helpful in connection with the making of tests, especially where there is suspicion of malingering. Thus, as table 1 shows, pectorals and forearm flexors are of nearly equal strength, as are thigh adductors and abductors, and the former groups are slightly less than twice as strong as the latter. In men of ordinary strength pectoral and forearm flexors are likely to range between 75 and 100 pounds, and thigh adductors and abductors between 40 and 60 pounds. Of fifty-five adult males selected at random, 80 per cent of the muscles of the short test fell within these limits.

That the proposed short test is practicable we believe these observations demonstrate. That it gives a reliable picture of the entire strength can reasonably be assumed from the fact that each individual muscle-group included in the test correlates well with the entire strength. One would expect to find that the summed strength of these several muscle-groups would correlate with the entire strength even better than do the individual muscle-groups. That this is the case was shown by a comparison, on the same series of adults on which the correlation of individual muscles were worked out, of the summed strength of the muscles of the short test with the entire strength. The coefficient of correlation of this comparison was 0.94 ± 0.01. So high a correlation constitutes sufficient demonstration that the short test is a reliable indicator of the entire strength.

Although the expression "entire strength" as here used applies actually only to that part of the strength represented by the muscle-groups of the complete test as outlined in table 1, these observations show that in all probability the real "entire strength" bears a fixed relationship to the strength of the muscles included in the complete test and also, therefore, to the short test. The principle of "random sampling" applies here. The muscle-groups included in the complete test constitute a fairly large sample of the whole musculature. Individual muscles and small groups of muscles correlate well with the summed strength of the complete-test (see above, also Martin: loc. cit., p. 72). There is no physiological or statistical principle that would justify any other assumption than that the muscle-groups not included in the complete test correlate equally well with it and with each other.

Muscular symmetry. In the first paper of this series (4) the distribution of strength among the muscles of the body in children was shown to vary somewhat with age so that three distinct age-groups could be established (loc. cit., p. 71). The symmetry of individuals was shown to deviate from the ideals as set down in the table by varying amounts, averaging for the entire series 16.7 per cent (loc. cit., p. 79, table). Application of the method there described to the present series of adult males gives the figures for percentage strength distribution that are presented in table 1 of this paper. These figures are averages of sufficient data except for plantar flexion and finger extension; the observations on which those values were obtained are fewer than desirable. Considerable errors in these two values would affect the others of the series only slightly, however, and it is felt that they are sufficiently close approximations for present purposes.

The deviations of individuals from ideal symmetry are decidedly less in this series of adult males than in the series of children presented in the former paper. The average mean deviation for this series is 10.7 as compared with 16.7 for the former. In fact, the greatest deviation in this series, a deviation of 16.5, is less than the average for children. We were quite unprepared to find adults more symmetrical as a class than children and are inclined to think that we may have happened upon an exceptionally symmetrical group. Most of them were college undergraduates and engaged at the time when these tests were being made in intensive military training.

The relation of strength to weight. A point on which stress was laid in the former paper (4) was that the ratio of strength to weight in children tends to be constant. That the same would hold for adults seems unlikely, chiefly because so many other factors in addition to weight become operative in adults in determining strength. In the former paper (p. 74) mention was made of the fact that boys of seventeen and eighteen years show higher strength-weight ratios than do younger boys, showing the beginnings of departure from the childish condition and suggesting the incidence of additional factors.

For a study of the relation of strength to weight we had available a series of one hundred and twenty-two adult males. This included our Stanford instructors and students, a few cases from the infantile paralysis series mentioned above, and a number of recent army recruits that we were enabled to test through the courtesy and coöperation of various army officers. The group as a whole would be considered as made up of outdoor men. The Stanford students, as previously noted, were most of them taking intensive military training. The weights ranged from 107 to 196 pounds, averaging 146.6. The strengths ranged from 2000 to 5800 pounds, averaging, in round numbers, 3900. (A single individual, the college strong man, weighing 208 pounds, made a strength record of 7600 pounds. This is so far in excess of our other records that we do not include it among our averages). The ratios of strength to weight ranged from 19 to 37, averaging 26.6. We are inclined to think that our figures for average strength and average strength-weight ratio are somewhat higher than they would be for adult males in general.

As would be expected, there is a moderate correlation between strength and weight, the coefficient for this series being 0.58 ± 0.04. This accords with the familiar fact that on the whole large men are stronger than small men. The correlation is not close enough, however, to

indicate that with adults, as with children, the body-weight is the dominant factor in determining strength. Whipple (loc. cit., p. 114) cites observations of correlation between strength and weight, in which strength was tested by a different method, with the following results: at Oxford, coefficient of correlation 0.46; at Cambridge, coefficient of correlation 0.56.

The significance of the strength-weight ratio. Where two individuals of equal weight differ widely in strength there are evidently at least four factors which may have influence in accounting for the difference. The first of these is actual amount of muscular tissue. There are undoubtedly considerable variations in the amounts of muscle substance present in the bodies of persons whose total weight is the same. A second factor is bodily configuration. It is quite conceivable that certain configurations lend themselves more favorably to effective exhibitions of muscular power than do others. Information on this point is lacking although it should be stated that in respect to the factor of height as an element in bodily configuration we have not been able to secure any evidence that it has significance. In investigating this point we grouped all our cases according to weight, using group intervals of five pounds, and then arranged all the members of each group in ascending order of height. We then examined the distribution of the strongest and next strongest members of each group with relation to height, and similarly of the weakest and next weakest. We found, however, nothing significant. One-half the strong were among the short men and the other half among the tall. Substantially the same distribution appeared also among the weak. Furthermore, the average strengths of the short and tall halves of each group were virtually equal.

A third factor is muscle-quality, and this undoubtedly has much to do with determining the strength. The fourth factor we may call, for lack of a better term, innervation. There can be little doubt that individuals vary in the extent to which they are able, by volition, to elicit muscular effort. That the usual manifestations fall far short of the potential maximum is shown by the familiar effects of excitement, as in the "strength of desperation." We have been at pains in the development of our procedure to avoid introducing the factor of excitement. The attempt has been to base the showing upon a maximal volitional effort made rather as a matter of routine than as a feature of competition or of desire to establish a record. Our feeling has been that a maximum effort made in "cold blood" gives a more uniform, and probably also a truer picture of the neuro-muscular power than would

a similar effort made under the stimulus of excitement. On the whole, muscle quality and innervation seem to us the factors most likely to dominate in the determination of strength, and in accordance with this view we are inclined to interpret high strength-weight ratios as indicative of good muscle-quality and good innervation and low strength-weight ratos as indicative of poor muscle-quality and poor innervation. Further investigation will be necessary before these factors can be separated; indeed it is quite within the bounds of physiological possibility that they are not separable; that excellence in one feature is always bound up with excellence in the other and vice versa.

Physical classification. For convenience in assigning individuals to categories in accordance with their physical strength, some simple scheme of classification is desirable. If our assumption is correct that the strength-weight ratio is an index of muscle-quality and of innervation it would suggest itself as a sound basis on which to work out such a classification. The limits of any such classification must be more or less arbitrary, at least until sufficient data are accumulated to enable them to be established by reference to a normal probability curve. If we are correct in our assumption that the averages of our present series are somewhat higher than would hold for adult males in general, the distribution of our cases about their average is not strictly comparable with the true distribution for adult males. We feel disposed, therefore, to suggest tentatively somewhat lower limits for our proposed classes than would be indicated by our data taken by themselves. The limits of the proposed classes are given in the subjoined table.

Class	*Strength-weight ratio*
A	more than 30
B	25.1–30
C	20.1–25
D	16.1–20
E	less than 16.1

The distribution of individuals in our series of 122 is as follows: class A, 17 (14 per cent); class B, 56 (46 per cent); class C, 47 (38 per cent); class D, 2 (2 per cent); class E, none. In general terms we would say that class A includes exceptionally strong men; class B, men of more than average strength; class C, men of average strength; class D, men of less than average strength; and class E, decided weaklings. Although our series included, according to this classification, no E men and only two D men, we think it altogether likely that a more representative

series, one in which clerks and factory hands were represented, would contain a due proportion of D men and a reduced percentage of A and B men. We are doubtful whether many healthy adult males will be encountered whose strength is so slight as to put them into the E class although our experience among the classes of the population in which marked physical weakness is likely to prevail is practically nil.

This classification, based on the strength-weight ratio, draws no distinction between small men of good quality and large men of equally good quality, although the latter will obviously be actually much more powerful than the former. For the practical purpose of assigning men to categories in accordance with their ability to achieve it would appear that some modification of the classification might well be made in which there is recognition of the importance of absolute muscular power as well as of good muscle-quality and good innervation. This end would be achieved if definite lower limits of strength were assigned to each class. Tentatively we would suggest the following limits: class A, 5000 pounds; class B, 4000 pounds; class C, 3000 pounds; class D, 1600 pounds. The practical effect of these absolute limits would be to require small men to show higher strength-weight ratios than large men, if they are to be placed in the higher classes. In no case would we reduce the limits suggested for the strength-weight ratios of the different classes. It would follow that a man weighing distinctly more than the average would have to show a strength well above the lower absolute limit of any given class in order to attain a strength-weight ratio that would admit him to it.

SUMMARY

1. The distribution of strength among the muscles in adult males is given (table 1).
2. The correlation between the strength of individual muscles and the entire strength is given (table 2).
3. Statistical evidence is presented showing that estimations of entire strength based on actual determinations of the strength of a few muscle-groups are valid.
4. Four pairs of muscle-groups: pectorals, forearm flexors, thigh adductors and thigh abductors are shown to correlate individually with entire strength to a satisfactory degree and to be also practically adapted for testing. They are selected, therefore, as constituting the "short" test.
5. The technique of the short test is presented in detail.

6. The summed strength of the muscles of the short test is shown to correlate well with the entire strength, the coefficient being 0.94 ± 0.01.

7. The adult males of this series are shown to have a higher average symmetry than the children of the former series; the average is 10.7 as compared with 16.7.

8. The ratio of strength to weight does not show the tendency to be constant in adult males that is seen in children. There is, however, a moderate correlation between strength and weight.

9. The factors influencing the strength-weight ratio are discussed. The conclusion is drawn that a high ratio signifies good muscle-quality and good innervation.

10. A physical classification, based primarily on the strength-weight ratio but modified to take some account of actual strength, is proposed.

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THE SIGNIFICANCE OF UNDISSOCIATED CARBON DIOXIDE IN RESPIRATION

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Received for publication June 22, 1918

The regulation of breathing has occupied the attention of physiologists for many years but the work of Haldane and Priestley (1) in 1905 marks an epoch in the development of our knowledge of the subject. The facts they presented at that time seemed to indicate that the CO_2 of the arterial blood afforded the adequate stimulus to the respiratory center and that breathing was so regulated as to maintain at a constant level the CO_2 pressure in the alveolar air and hence in the arterial blood. It was well known, however, at the time this hypothesis was advanced that not only CO_2 but also other acids excited the respiratory center when they were added to the blood. As a result of his perfusion experiments on newborn rabbits, Winterstein (2) in 1911 formulated the hypothesis that the blood C_H was responsible for the regulation of breathing. More convincing evidence for this conclusion was given a year later by the work of Hasselbalch and Lundsgaard (3). They were able to show a definite relationship between CO_2 pressure and C_H in blood. The further work of Hasselbalch (4) on the effect of diet showed that the alveolar CO_2 pressure may be altered by several millimeters, but that the change was in such a direction as to maintain the C_H of the blood appreciably normal. These results and others led Hasselbalch to the conclusion that pulmonary ventilation is so adjusted as to maintain the C_H of the blood constant and that CO_2 excites the respiratory center only by virtue of its acting as an acid when in solution.

The work of Hasselbalch and Lundsgaard was generally accepted by the British school of physiologists led by Haldane and it has gained further support by the more recent clinical observations on the acid intoxications of diabetes and nephritis. Indeed most of the experimental and clinical data at present available seem to substantiate the

hypothesis that the hydrogen ion is the true respiratory hormone. A certain security is given to this view by the nature of the relationship between CO_2 tension and C_H in blood. In the intact animal it is difficult to elicit evidence in favor of the specificity of CO_2 which might not also be attributed to the C_H. To circumvent this obstacle, highly artificial methods such as perfusion experiments have been employed by the few investigators who have attempted to show that CO_2 might act as a specific respiratory hormone. Lacqueur and Verzar (5), using Winterstein's original method of perfusing young rabbits, obtained some evidence in support of this view but their results have not been accepted. More recently Hooker (6) and his collaborators, by perfusing the isolated medulla of dogs, have found that bloods of a certain C_H with a high CO_2 tension excite the respiratory center more than blood of the same C_H containing HCl with a low tension of CO_2. The question naturally arises whether or not the results found with the very artificial preparation which these workers used would correspond to those obtained in the intact animal, in which the various tissues are concerned with the maintenance of the normal acid-base equilibrium of the body fluids. Also the criticism might be made that the C_H of the perfusate should be determined after the perfusion as well as before it. In spite of these objections the observations of Hooker are suggestive and correspond in a general way with the results reported in this paper.

At the present time there are many facts indicating that one of the important functions of respiration is to maintain at a constant level the ratio $\left(\frac{H_2CO_3}{HCO_3}\right)$ and hence the C_H of the blood. Any change to the acid side stimulates respiration. The tension of CO_2 during its excretion is varied, which serves as a very delicate mechanism to regulate the reaction of the blood. So far as this function of the respiratory center is concerned there is no doubt. But may the center not be affected by certain changes in the chemical composition of the blood even though there is no consequent elevation in the C_H, such for example as an increased CO_2 tension in the presence of a true alkalosis?

It is generally believed that an accumulation of free CO_2 in the blood is deleterious to the organism only in so far as it elevates the C_H, but the observations here reported show that the respiratory center resists any abnormal elevation of CO_2 tension even though the blood supplied to the medulla is distinctly more alkaline than normal. To prove this point it is of course desirable to work with the intact animal

under conditions as nearly within physiological limits as possible. On the other hand, the blood must be rendered sufficiently alkaline so that when the CO_2 tension is raised the resulting change in C_H will still fall on the alkaline side of normality.

It has long been taught that the injection of alkali into an animal produces apnea. However this depends not only on the strength of the alkali but chiefly on its rate of injection. The arterial blood of an animal may be made as alkaline as P_H 7.8, yet the respiration continues in a perfectly normal fashion. In fact the tidal air per minute may be the same within a few cubic centimeters whether the P_H of the blood is 7.4 or 7.8. In such a preparation the CO_2 tension may be elevated while the C_H is appreciably more alkaline than normal. The resulting effect on the respiration cannot be attributed to any abnormal elevation in the blood C_H acting as a stimulus to the respiratory center.

Methods. Decerebrate cats were used in all the experiments reported in this paper. In order to obtain a normal response of the respiratory mechanism to a given stimulus, it is essential to avoid the depressing effects that all general anesthetics have on the center. This point was emphasized by the writer in a previous communication (7). As we have repeatedly observed, a given stimulus may evoke a definite respiratory response in the unanesthetized animal, whereas the same stimulus in an anesthetized animal produces little or no effect.

After decerebration,[1] an interval of about forty-five minutes was allowed for the elimination of the ether used before the operation. All animals that did not lie perfectly quiet or which had any irregularity in respiration were discarded. Blood samples were taken directly from either the femoral or the carotid artery with care exercised to avoid the loss of CO_2. The P_H determinations were done with the dialysis indicator method, using phenolsulphonephthalein as indicator. This method has recently been checked against the gas-chain by Clark and Lubs (8), who found that it gave results in close agreement with electrometric determinations. The results for the total CO_2 content of the blood were obtained with a modified Barcroft-Haldane apparatus, which was determined to be accurate within 1.5 per cent. The air analyses were made with the well known Haldane apparatus.

Technique of experiments. After decerebration and while the animal was respiring room air, samples of blood were taken for the P_H and total

[1] Details of the method of decerebration used have already been described (7).

CO_2 determinations. The animal was now made to breathe in a closed system through valves separating the inspired from the expired air. In the system was a graduated Gad spirometer equipped with a writing style to record on a kymograph. Together with a chronograph tracing there was thus obtained the exact amount of tidal air per minute corresponding to increasing percentages of CO_2. Samples of the air from the bottle out of which the animal inspired were taken at frequent intervals and analyzed for CO_2. Oxygen was run into the system at such a rate as to maintain its concentration at approximately 20 per cent. When the CO_2 in the inspired air had risen to about 5 per cent, samples of blood were taken and the P_H and total carbonate content determined. These results together with the respiratory records were used for comparison with similar data obtained from the same animal after the injection of alkali.

After securing what was considered under the experimental conditions to be the normal reaction to CO_2, the animal was allowed to breathe room air again for about forty-five minutes. Alkali (0.35 grams Na_2CO_3 per kilogram) was then injected into the femoral vein at the rate of 0.25 cc. of a 5 per cent solution per minute. By experience it has been found that this quantity of Na_2CO_3 when injected at the rate indicated does not disturb the respiration and will invariably change the P_H from a normal 7.4 to 7.7 or 7.8.

At the end of the injection P_H and total CO_2 determinations were made on the blood. A rebreathing experiment was now done and when the inspired CO_2 had reached about 5 per cent, blood samples were again taken. It is apparent that the experimental procedure differed in no way from that described above, the object here being to ascertain the effect of alkalosis on the reaction to CO_2. Typical records of the respiratory response to CO_2 are shown in figure 1. These tracings were obtained from the same animal, the upper row before alkali and the lower row after alkali.

In another series of observations a slightly different technique was followed. Here instead of accumulating CO_2 by rebreathing, the alkalinized animal was made to breathe a CO_2-rich air 6 to 7 per cent at once. After about one and one-half minutes, and while still inspiring this mixture, blood was taken for the P_H. This type of experiment shows in a more striking way the stimulating effect of CO_2 on respiration (see fig. 2).

Results. All results are expressed in tabular form. In table 1 are given the data obtained in the first group of experiments, comparing

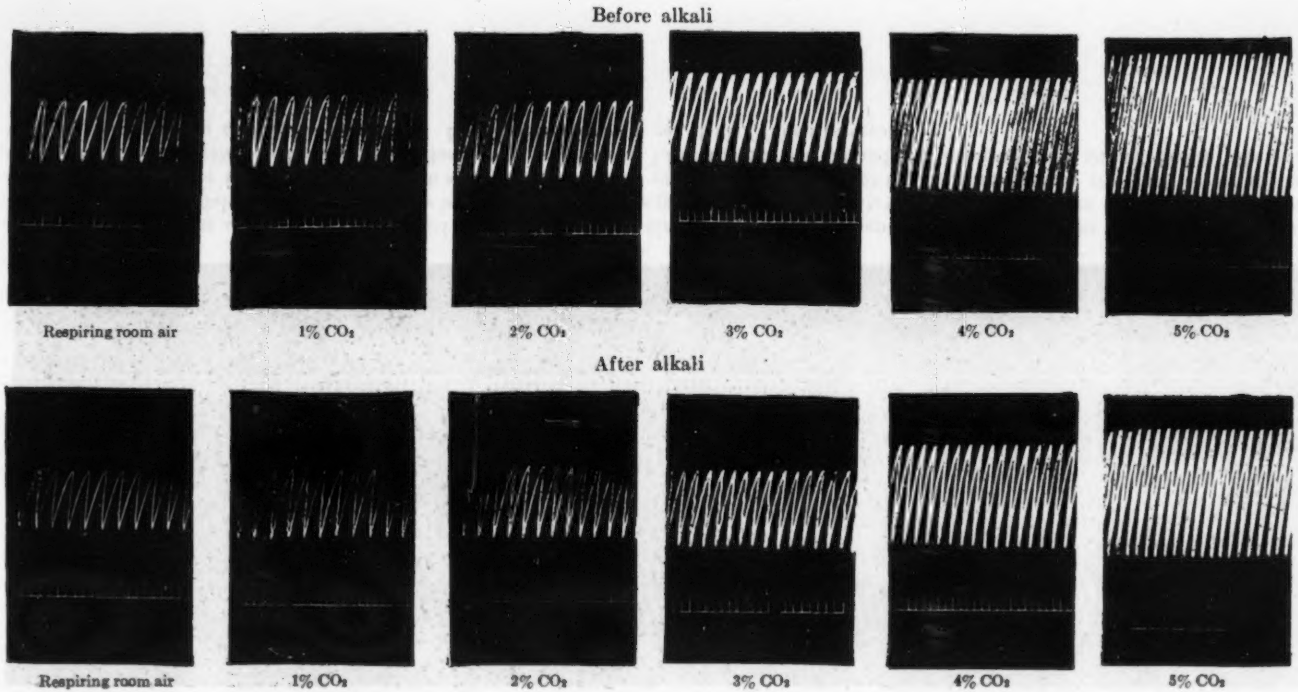

Fig. 1. Showing the respiratory records obtained in experiment 32. Upper row of tracings represents the tidal air in the normal animal corresponding to increasing percentages of inspired CO_2. The lower row was taken under like conditions from the same animal after the injection of alkali (0.35 gm. Na_2CO_3 per kg.) Note the similarity of the response in the two cases. Upstroke = expiration. Each millimeter = 4 cc. of air. Time in seconds.

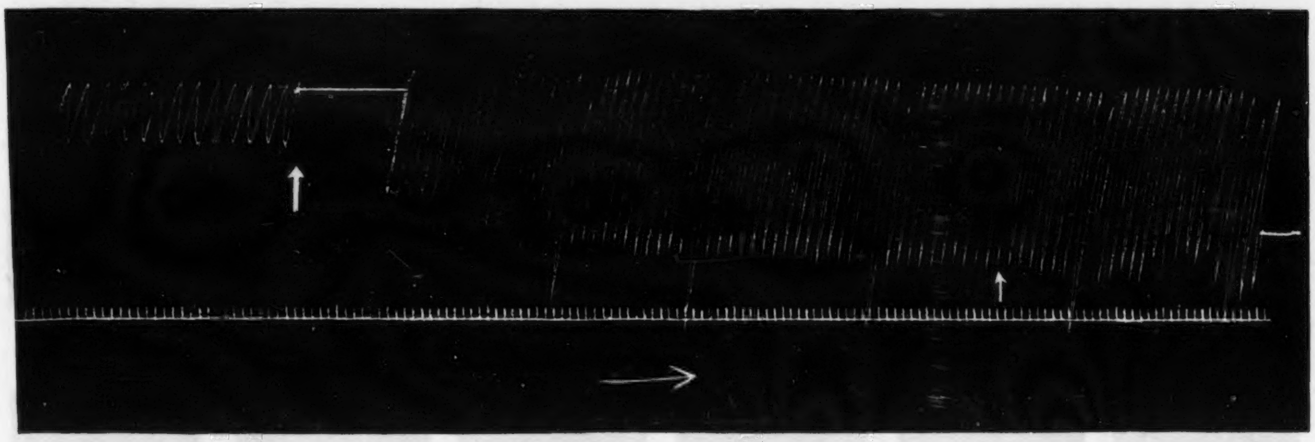

Fig. 2. Showing the effect in the alkalinized animal of inspiring air containing 7.2 per cent CO_2, (exp. 37). The first part of the tracing represents the tidal air while room air was being respired. At the pause in the tracing CO_2 was introduced. Note the stimulating effect on respiration. After inspiring the CO_2 rich air for about one and one-half minutes and at the point marked by the small arrow to the right, a sample of carotid blood was taken. The P_H was found to be 7.55, whereas the normal for this particular animal before alkalosis was 7.4. Upstroke in tracing = expiration. Each millimeter = 4 cc. of air. Time in seconds.

TABLE 1

The reaction to CO_2 in the normal and alkalinized animal

EXP. NO.	INSPIRED AIR	BEFORE ALKALI				AFTER ALKALI			
		Respiratory rate per minute	Tidal air per minute	P_H of arterial blood	Total CO_2 arterial blood per 100 cc.	Respiratory rate per minute	Tidal air per minute	P_H of artorial blood	Total CO_2 arterial blood per 100 cc.
			cc.		cc.		cc.		cc.
32	Room air..........	28	1,008	7.4	38	31	1,054	7.8	76
	1 per cent CO_2.....	30	1,260			31	1,209		
	2 per cent CO_2.....	30	1,380			32	1,344		
	3 per cent CO_2.....	35	1,960			35	1,610		
	4 per cent CO_2.....	43	3,182			38	2,356		
	5 per cent CO_2.....	50	4,400	7.2	56	46	3,496	7.5	87
33	Room air..........	29	928	7.35	36	29	940	7.75	70
	1 per cent CO_2.....	32	1,408			30	1,200		
	2 per cent CO_2.....	34	2,040			33	1,430		
	3 per cent CO_2.....	39	2,656			35	1,780		
	4 per cent CO_2.....	43	3,612			38	2,420		
	5 per cent CO_2.....	45	4,500	7.25	60	42	3,360	7.60	92
34	Room air..........	26	728	7.4	40	27	735	7.8	72
	1 per cent CO_2.....	27	820			27	800		
	2 per cent CO_2.....	29	1,000			28	870		
	3 per cent CO_2.....	30	1,640			30	1,225		
	4 per cent CO_2.....	34	2,230			32	1,640		
	5 per cent CO_2.....	37	3,256	7.25	59	34	2,183	7.55	90
35	Room air..........	24	1,056	7.4	35	26	1,000	7.70	73
	1 per cent CO_2.....	25	1,142			26	1,092		
	2 per cent CO_2.....	27	1,543			27	1,154		
	3 per cent CO_2.....	31	2,345			29	1,462		
	4 per cent CO_2.....	34	3,280			33	2,010		
	5 per cent CO_2.....	37	4,562	7.20	54	35	2,860	7.55	85
36	Room air..........	26	708	7.35		25	722	7.75	
	1 per cent CO_2.....	27	822			25	761		
	2 per cent CO_2.....	29	940			27	830		
	3 per cent CO_2.....	32	1,320			29	931		
	4 per cent CO_2.....	36	2,180			32	1,540		
	5 per cent CO_2.....	44	3,120	7.25		36	2,132	7.6	81

the reaction to CO_2 before and after alkali. The respiratory rate and and tidal air per minute corresponding to various percentages of inspired CO_2 were taken from tracings similar to the ones shown in figures 1 and 2. In table 2 are given the values for the free and combined CO_2 in the blood in the normal and alkalinized animal, first while breathing room air and again while inspiring 5 per cent CO_2. Although not representing absolute values,[2] these data are valuable

TABLE 2

The relation between the free and the combined CO_2 in the blood before and after alkali

EXP. NO.	RESPIRED AIR	BEFORE ALKALI				AFTER ALKALI			
		P_H	$(H_2CO_3)+(NaHCO_3)$	(H_2CO_3)	$(NaHCO_3)$	P_H	$(H_2CO_3)+(NaHCO_3)$	(H_2CO_3)	$(NaHCO_3)$
			cc. per 100 cc.	*cc. per 100 cc.*	*cc. per 100 cc.*		*cc. per 100 cc.*	*cc. per 100 cc.*	*cc. per 100 cc.*
32	Room air	7.4	38	2.0	36.0	7.8	76	1.6	74.4
	5 per cent CO_2	7.2	56	4.5	51.5	7.5	87	3.6	83.4
33	Room air	7.35	36	2.1	33.9	7.75	70	1.7	68.3
	5 per cent CO_2	7.25	60	4.3	55.7	7.6	92	3.1	88.9
34	Room air	7.4	40	2.1	37.9	7.8	72	1.5	70.5
	5 per cent CO_2	7.25	59	4.2	54.8	7.55	90	3.3	86.9
35	Room air	7.4	35	1.8	33.2	7.75	73	1.7	71.3
	5 per cent CO_2	7.2	54	4.3	49.7	7.55	85	3.2	81.8
36	Room air	7.35				7.75			
	5 per cent CO_2	7.25				7.6	81	2.7	78.3

in showing the relative changes which occur in the $\frac{H_2CO_3}{NaHCO_3}$ ratio of the blood under the experimental conditions. The results of the second series of more acute experiments are given in table 3. Here the respiratory data were obtained from tracings similar to the ones shown in figure 2.

[2] These results were calculated from the equation $\frac{H_2CO_3}{NaHCO_3} = \frac{\lambda C_H}{K}$, in which the known values of the constants were those given by Michaelis and Rona (1912), $K = 4.4 \times 10^{-7}$, λ for blood conditions $= 0.605$.

Discussion of results. The data obtained before the injection of Na_2CO_3 are similar to those previously reported (7) and are presented here for comparison to show in what way a condition of alkalosis affects the reaction to CO_2. It is noted that when a 5 per cent CO_2 air is inspired, the amount of CO_2 given off by the blood is markedly elevated above normal[3] while the C_H is detectably raised above the value found when room air was being respired. Under these experimental conditions with a partial pressure of CO_2 not above 50 mm., it is probable that the increased amount of CO_2 found in the blood was for the most part bound as $NaHCO_3$, whereas a relatively small amount was carried by the hemoglobin and other blood proteins. Buckmaster

TABLE 3

The effect of inspiring a CO_2-rich air for one and one-half minute on respiration and the P_H of the arterial blood in the alkalinized animal

EXP. NO.	INSPIRED AIR	RESPIRATORY RATE PER MINUTE	TIDAL AIR PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR PER MINUTE	P_H OF ARTERIAL BLOOD	P_H OF ARTERIAL BLOOD BEFORE ALKALI
37	Room air	32	1,152		7.8	7.4
	CO_2 = 7.2 per cent	44	4,928	327	7.55	
38	Room air	24	1,056		7.75	7.35
	CO_2 = 7.5 per cent	46	5,336	465	7.50	
39	Room air	28	840		7.75	7.4
	CO_2 = 6.4 per cent	40	4,182	400	7.60	
40	Room air	30	983		7.70	7.4
	CO_2 = 6.2 per cent	43	4,380	350	7.55	

(9), for example, has found that hemoglobin is capable of combining with more CO_2 than the other proteins found in blood. His figures show that at pressures between 760 and 70 mm., hemoglobin can bind considerable quantities of CO_2. But there is no conclusive evidence to indicate that this is true at the pressure of CO_2 which may obtain during life.

[3] Recently Henderson and Haggard (Journ. Biol. Chem., 1917, xxxiii, 343) have confirmed our earlier observations by showing that the total CO_2 content of the blood is raised in a condition of CO_2 acidosis, brought about either by breathing a CO_2-rich air or by depressing the respiratory mechanism with the administration of morphine.

A very significant fact is noted in the behavior of the alkalinized animal while breathing room air. Neither the respiratory rate nor the tidal air per minute is affected to any appreciable extent by the carbonate injection, although the P_H of the blood has been changed on the average from 7.4 to 7.8. This fact indicates that a given change in P_H on the alkaline side is attended with fewer apparent disturbances than a similar change to the acid side of normality. *However, the ability of the organism to tolerate a given elevation in C_H probably depends on the acid responsible for the elevation.* Certain observations that we have made on CO_2 acidosis have indicated that the physiological limits of C_H toward the acid side are greater for H_2CO_3 than for other acids. For example, the P_H of the arterial blood in a decerebrate cat may be changed from a normal 7.4 to 7.1 by breathing air rich in CO_2. After breathing room air for twenty minutes the blood P_H and respirations have returned to normal. This acute experiment may be done as often as three times in two hours without any apparent ill effects. On the other hand, it has been found extremely difficult to reduce the P_H to 7.1 by the slow injection of acids such as N/20 HCl or N/20 acetic. In the majority of cases the animals have developed convulsive movements and died before that level was reached.

One important factor concerned in the explanation of these results is the behavior of the body bicarbonate in the two cases. We have shown that the bicarbonate content of the blood is much increased in a condition of CO_2 acidosis. For example, the total CO_2 in the arterial blood was found to increase from a normal 38.4 vols. to 77.2 in a decerebrate cat whose blood P_H was lowered from 7.4 to 7.1 by breathing air rich in CO_2 (7). On the other hand Van Slyke (10) found that after the injection of 75 cc. N/1 (NH_2SO_4) into a dog, the P_H of the arterial blood had changed from a normal 7.33 to 7.17, while the total CO_2 was diminished from 38.7 to 10.1, a reduction of over 200 per cent. These facts have much significance. They show that in the two general types of acidosis, there is a wide variation in the amount of available alkali in the blood. In CO_2 acidosis the increased quantity of $NaHCO_3$ present in the blood serves as available alkali to neutralize any further acidity. In the case of other acids the situation is different. Here the alkali is consumed and instead of actually increasing in amount it undergoes a progressive diminution in proportion to the amount of acid present. In so far then as the available alkali of the blood is used up, the ability to withstand any further acidity must be diminished. Doubtless similar circumstances prevail in the tissues,

where it becomes increasingly difficult to maintain the environment within physiological limits of P_H as the bicarbonate content of the body fluids becomes depleted. These considerations alone, apart from the easy elimination of CO_2 by the lungs, indicate that the organism is better equipped to resist an increase in C_H due to H_2CO_3 than it is one caused by other acids.

A CONDITION OF TRUE ALKALOSIS NOT ACCOMPANIED BY APNEA

The absence of any respiratory disturbance accompanying a condition of true alkalosis indicates that there is not the close parallelism existing between the activity of the respiratory center and the C_H of the blood that has generally been supposed. Otherwise the degree of alkalinity present should give rise to apnea. Since the respiration continues in a normal way, it must be concluded that other factors besides the C_H may be concerned in the chemical regulation of breathing, and in this connection the free CO_2 of the blood deserves consideration.

Reference to table 2 shows that a definite increase in the blood alkalinity with only a slight change in the free CO_2 may be produced by the slow injection of Na_2CO_3. Under these circumstances the animal breathes normally. On the other hand, as is well known, apnea results when any appreciable alkalosis is caused by the methods ordinarily used, i.e., the rapid injection of alkali or superventilation of the lungs. Both procedures cause a sudden lowering in the CO_2 tension of the blood. It is therefore apparent that two types of alkalosis may be distinguished: *a*, having a normal CO_2 tension with no disturbance in breathing, as illustrated by the experiments here reported; *b*, having a lowered CO_2 tension accompanied by apnea.

The significance of a lowered CO_2 tension as the cause of apnea has been recognized for many years. Bieletzky (11) in 1882 showed that apnea was produced in birds by over-ventilation of the lungs. Later Fredericq (12), using his crossed cephalic circulation method, and Hougardy (13), by the injection of alkali, concluded that apnea was due to a diminution in the CO_2 tension of the arterial blood. A similar conclusion was reached by Campbell, Douglas, Haldane and Hobson (14) as a result of their experiments on man. They, however, interpreted their findings to accord with the hypothesis of Winterstein and Hasselbalch, attributing a preponderating influence to the C_H as the respiratory hormone. By so doing they assume that CO_2 affects the respiratory center only by virtue of its acid nature when in solution.

Accordingly a condition of alkalosis should be accompanied by apnea, which should persist until the metabolic CO_2 accumulated to such a degree as to raise the C_H to the threshold-exciting value for the respiratory center. The evidence here presented shows that this is not the case. Respiration continues regularly in the presence of a demonstrable alkalosis, provided the CO_2 tension in the blood is maintained about normal. On the other hand, we have found that apnea is very readily produced in the alkalinized animal by lowering the CO_2 tension with artificial ventilation. It is therefore clear that the apneic pause is not caused by a diminution in the blood C_H, as would be the case if the chemical regulation of breathing depended solely on blood reaction. Our results indicate a close association between apnea and CO_2 tension, thus corroborating the observations of earlier investigators. A certain significance is hereby given to CO_2, which will obviously be neglected so long as its effect on the respiratory center is considered to be an indirect one through the blood reaction.

It has been noted that the very slow injection of Na_2CO_3 has no effect on the respiration. A similar observation was made by Hougardy (13) but there was no method available at that time to determine the C_H of the blood. Reference to table 2 shows that the injection of 0.35 grams Na_2CO_3 per kilogram produces on the average a change in P_H from a normal 7.4 to 7.7 or 7.8, whereas the free CO_2 in the blood undergoes only a slight diminution. In view of the fact that Na_2CO_3 is capable of fixing CO_2 to form $NaHCO_3$, the question naturally arises concerning the mechanism by which the organism is able to maintain an approximately normal CO_2 tension in the arterial blood. Doubtless an important factor in this connection is the retention of a considerable quantity of metabolic CO_2. It is apparent that such a mechanism would afford the most available means of combating an impending alkalosis. In experiment 32, table 2, the normal total CO_2 was 38 vol. per cent; after the alkali injection it was 76 vol. per cent. Only about one-third of this increase found can be derived from the CO_2 contained in the quantity of Na_2CO_3 injected. For example 0.35 gram Na_2CO_3 contains 0.145 gram CO_2. Estimating the body fluids at 700 cc. per kilogram body weight, the total CO_2 found after the carbonate injection is equivalent to 0.476 gram CO_2 at 20°—760 mm. This value is based on the assumption that the slow rate of injection permitted a uniform distribution to the body fluids. Although we have no data on the total respiratory exchange, in all probability the disparity found is due to a retention of CO_2 that might otherwise be eliminated by the

lungs. When an alkali capable of combining with CO_2 is rapidly injected, the most ready means of neutralization is by the free CO_2 in the blood. This naturally undergoes a sudden diminution, which no doubt explains the apneic pause under these circumstances. On the other hand, the slow injection of alkali allows sufficient time for the retention of metabolic CO_2 so that the free CO_2 of the blood suffers no marked diminution and consequently the breathing is unaffected.

THE EFFECT OF ALKALOSIS ON THE REACTION TO CO_2

Reference to table 1 and figure 1 shows that the general character of the respiratory response to CO_2 is the same after the injection of alkali as before. A slight difference, however, is noted in the quantity of tidal air which corresponds to a given percentage of inspired CO_2. This disparity is shown graphically in figure 3. Here the curves[4] were plotted from the data in table 1, and therefore represent the mean in five animals before and after receiving alkali. It is noted that similar percentages of CO_2 elicit a slightly more vigorous reaction on the part of the respiratory center before alkalosis is established. Furthermore, this disparity becomes more conspicuous with increasing quantities of CO_2 in the inspired air. The following facts merit consideration in explaining these phenomena: *a*, the levels at which the C_H changes; and *b*, the difference in the buffer value of the blood in normal and alkalinized animals. Reference to table 3 indicates that when the normal animal is made to breathe increasing percentages of CO_2 up to 5 per cent, both the CO_2 tension and the C_H of the blood are elevated. In this case the respiratory center may be influenced by two factors, CO_2 and C_H. After alkalosis is established only the CO_2 tension is raised by breathing 5 per cent CO_2, while the C_H always remains on the alkaline side of the normal. It is not surprising under these conditions that CO_2 might elicit a less vigorous respiratory response. In other words, the threshold value of CO_2 may be raised in a condition of alkalosis and lowered in the presence of an elevation in the C_H of the blood.

It is observed that the total CO_2 content of the arterial blood is raised by approximately 100 per cent after the injection of the quantity of Na_2CO_3 used. Consequently the buffer value must be greatly increased. The addition of a given quantity of CO_2 to such a blood will cause less elevation in the H_2CO_3 content than would be the case with

[4] The data from single experiments give curves of the same general contour.

normal blood. This is shown by the data in table 2. While the alkalinized animal is breathing room air, the free CO_2 is a little under the value found before the alkali injection. A similar disparity is noted when air containing 5 per cent CO_2 is inspired. From these considerations it may be concluded that both the increased buffer value and

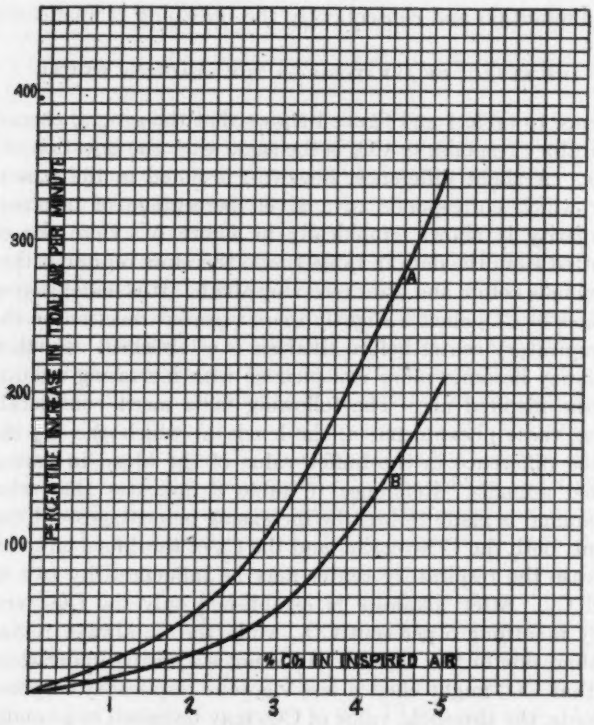

Fig. 3. Composite curves from the data obtained in five animals (see table 1) showing the respiratory reaction to increasing percentages of CO_2 in the inspired air. *A*, the normal reaction before alkali injection. *B*, after the intravenous injection of Na_2CO_3 (0.35 gram per kg.).

the diminution in C_H of the blood are concerned in the explanation of the fact that increasing percentages of CO_2 in the inspired air cause a somewhat less vigorous respiratory response in the alkalinized than in the normal animal. Otherwise the similarity of the response is very striking in the two cases (see fig. 3).

Table 3 shows the results obtained in the series of acute experiments in which the alkalinized animal was made to breathe a CO_2-rich air instead of accumulating the CO_2 by rebreathing. The respiratory data were obtained from tracings similar to the one shown in figure 2. When the inspired CO_2 was suddenly changed from 0.04 per cent (room air) to 6 or 7 per cent, there was a marked stimulation of the respiratory center, evidenced by an increase in the rate as well as the depth of breathing. Under these circumstances the tidal air per minute was elevated in the different animals, ranging from 327 to 465 per cent. It is noted that this marked augmentation in respiration occurred in the presence of a blood P_H that was demonstrably on the alkaline side of the normal found before alkalosis was established. Such results afford a striking illustration of the stimulating effect of CO_2 on respiration, which obviously cannot be attributed to any abnormal elevation in the C_H of the blood.

The current hypothesis recognizing the C_H as the sole factor concerned in the chemical regulation of breathing has come to be so universally accepted that little attention is paid to the effect of undissociated CO_2 on respiration. It is taught that CO_2 can influence the respiratory center only by virtue of its acid properties when in solution. Consequently an abnormal elevation in CO_2 tension in the blood is thought to stimulate respiration only in so far as the C_H is thereby raised. To quote from a recent contribution of Haldane (15),

> . . . . there seems now to be no doubt that it is true that what the respiratory center responds to is hydrogen ion concentration and not mere CO_2 pressure.

In the light of present facts there is no doubt that the C_H is one important factor influencing breathing. For example, it is well established that under normal conditions pulmonary ventilation is so regulated as to keep the C_H appreciably constant while any change to the acid side is accompanied by a marked increase in respiration. It has therefore been concluded that a close parallelism existed between pulmonary ventilation and the blood C_H. However, when we compare the activity of the respiratory center at slightly alkaline levels of C_H we find that no such definite relationship exists. Here instead of apnea resulting, as might be expected, the breathing continues regularly and is apparently directed toward maintaining the CO_2 at a normal level. Any elevation in this level causes a marked stimulation of respiration.

In view of these facts it seems probable that the so-called respiratory hormone is not a single entity in the form of the hydrogen ion as postulated by the theory of Winterstein and Hasselbalch. To assume that the respiratory center is oblivious to changes in the chemical equilibria in the blood except in so far as such changes may alter the CH, is placing a limitation on its function which is hardly commensurate with the importance of respiration in the living animal. Apparently the center is very sensitive to undissociated CO_2, and certainly in the alkalinized animal our results indicate that CO_2 may act as a specific respiratory hormone. This gives an added importance to the physiological significance of CO_2 and at the same time illustrates a function of the respiratory center which will not be appreciated if we restrict its activity solely to the influence of the C_H.

SUMMARY

The reaction to CO_2 has been compared in the normal and alkalinized animal, using the quantitative response of respiration, together with the P_H and total carbonate content of the arterial blood in the two cases. By the slow injection of Na_2CO_3 (0.35 gram per kilogram), a demonstrable alkalosis is produced, yet the breathing continues normal indicating that a change in the C_H to the alkaline side is better tolerated than a similar change toward acidity. Examination of the arterial blood shows that by the alkali injection the P_H has been altered from the normal 7.4 to 7.8, while the total CO_2 content is elevated by approximately 100 per cent. Evidence is presented to show that the regular type of breathing seen in the alkalinized animal is intimately associated with the maintenance of the free CO_2 in the blood at a normal level, while any appreciable elevation in this level causes a marked stimulation of respiration in spite of the fact that the C_H of the blood is sensibly on the alkaline side of normality. Such observations have led to the following conclusions:

1. There is not the close parallelism between respiration and the C_H of the arterial blood supposed by the theory of Winterstein and Hasselbalch.
2. At slightly alkaline levels of C_H, pulmonary ventilation is so regulated as to maintain the CO_2 tension in the blood normal.
3. A condition of true alkalosis is accompanied by apnea only when the method used to lower the C_H causes at the same time a sudden fall in the CO_2 tension; as, for example, the rapid injection of an alkali capable of binding CO_2, or by superventilation of the lungs.

4. Undissociated CO_2 acts as a specific respiratory hormone; therefore the physiological effects of CO_2 on respiration can not be attributed solely to its acid properties when in solution.

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XIV. DIFFERENCES IN THE CATALASE CONTENT OF MUSCLE FROM DIFFERENT PARTS OF THE STOMACH

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Received for publication July 9, 1918

Catalase is the ferment which liberates oxygen from hydrogen peroxid. As we have shown in previous papers, it seems to have something to do with the oxidative processes. Our work on the muscle of the small intestine suggested strongly that the catalase content of a tissue can be used as an index of its metabolic activity (1). We found that the gradient of catalase content from duodenum to ileum closely paralleled the gradients of rhythmicity, irritability, latent period and CO_2 production.

Alvarez has shown that the peristaltic waves in the stomach probably follow gradients of irritability, rhythmicity and latent period from cardia to pylorus (2). We wish to show in this paper that the catalase content of the muscle is also graded from cardia to pylorus.

TECHNIC

This has been described carefully in a previous paper (1). The animals were killed either by a blow on the head or by chloroform and bleeding. By using a shaking machine five tests on muscle from the same stomach were run at the same time under identical conditions. In the following tables the figures represent cubic centimeters of oxygen at atmospheric pressure, liberated from 15 cc. of hydrogen peroxid by the catalase in 0.3 gram of minced muscle. The muscle was peeled off of the mucous membrane. No difficulty was met with in doing this except along the lesser curvature of the rabbit's stomach. There the mucosa had to be scraped off. In some of the earlier experiments the hydrogen peroxid was not neutralized.

Lesser Curvature

	H₂O₂ NEUTRALIZED						AVERAGES
Cat							
1. Near cardia	43.0	52.4	44.0	36.2	40.4	35.3	41.9
2.	38.3	42.4	39.5	33.1	39.2	39.4	38.7
3.	30.3	39.0	32.8	35.3	35.4	25.8	33.1
4. Near pylorus	29.6	31.6	36.5	31.9	28.3	23.1	30.2
Dog							
1. Near cardia	23.5	19.5	18.6	26.8	25.2		22.7
2.	17.5	14.4	17.4	15.1	14.7		15.1
3.	17.8	16.4	16.8	11.7	11.4		14.8
4. Near pylorus	8.2	17.6	18.8	11.3	10.0		14.4
	H_2O_2 NOT NEUTRALIZED						AVERAGES
Rabbit							
1. Near cardia	25.0	22.5	25.0	31.5	25.0	28.5	26.3
2.	19.9	22.5	28.3	20.9	28.7	29.0	24.9
3.	18.7	16.2	18.4	15.3	25.2	28.7	20.4
4. Near pylorus	16.0	12.9	19.0	14.0	22.5	25.4	18.3

Greater Curvature

Note: For purposes of comparison a test was made also of the catalase in the muscle from the first part of the duodenum.

	H_2O_2 NEUTRALIZED								AVERAGES
Cat									
1. Near cardia	32.6	38.1	57.7	56.4	40.4	49.6	42.8	55.3	44.1
2.	30.9	28.0	48.2	54.8	38.8	35.9	39.4	51.2	40.9
3.	27.4	40.8	43.7	39.1	31.5	41.3	36.8	45.3	38.2
4. Near pylorus	22.4	41.8	44.1	49.2	27.2	32.5	34.3	52.0	37.8
5. Duodenum	24.4	34.7	43.0	46.7	41.0	48.3	32.4	48.6	39.9
Dog									
1. Near cardia	24.0	23.5	26.1	17.4	17.0	19.0			21.2
2.	20.7	22.6	18.8	14.9	15.6	18.5			18.1
3.	29.4	25.9	18.9	16.6	16.2	18 4			20.9
4. Near pylorus	30.5	21.0	10.5	14.8	14.8	22.1			18.9
5. Duodenum	25.2	25.0	23.3	22.2	16.9	21.6			22.4
	H_2O_2 NOT NEUTRALIZED								AVERAGES
Rabbit									
1. Near cardia	17.4	17.0	21.0	17.7	18.8	15.0	15.9	14.7	17.2
2.	14.9	15.6	18.2	11.4	19.9	18.9	16.5	15.0	16.3
3.	16.6	16.2	19.5	17.1	22.0	13.0	15.6	13.6	16.7
4. Near pylorus	14.8	14.8	14.1	14.8	15.9	10.5	18.2	12.4	14.4
5. Duodenum	22.2	16.9	21.3	33.3	18.6	21.9	18.2	15.0	20.9

DISCUSSION

A glance at figure 1 shows the definite gradation in the catalase content from cardia to pylorus. This is most marked along the lesser curvature in the cat and rabbit. The irregularity of the curves in the dog may be due partly to the difficulties encountered in getting perfectly healthy dogs. Animals with definite distemper, large numbers of intestinal parasites or diarrhea had even more irregular curves.

The poorer gradation along the greater curvature in all the animals is to be expected. The remnants of the primitive intestinal tube are along the lesser curvature while a considerable proportion of the greater curvature develops in the embryo as a cecum (3). The curves from the rabbit's stomach conform very closely to what we expected from the previous studies on the latent period, irritability and rhythmicity of the muscle. It is hard to say why in the cat and dog the figures should be higher on the greater curvature, where the rates of contraction were slower. Another thing which is hard to explain is the great difference between the amounts of catalase on the two curvatures near the pylorus in the cat and dog. To be sure, some differences in latent period and irritability were found in different parts of the antrum but when we think of the development and function of this part of the stomach there would seem no need for any differentiation in the muscle along the two curvatures. In order to get a little more light on this subject, estimations were made on equal weights of muscle from the two curvatures and from the anterior and posterior surfaces of the antrum. The following figures from the cat and dog show a striking difference between the catalase content of the muscle on the lesser curvature and that in the rest of the antrum.

	CAT	DOG
Lesser curvature	32.0	21.8
Anterior surface	40.2	32.3
Posterior surface	43.3	33.8
Greater curvature	47.2	43.2

Another interesting point is that the pace-making area near the cardia had a much higher catalase content than that of the pyloric region in which most of the muscular work of the stomach is done. It appears from this that the amount of catalase in a muscle depends

not so much upon the amount of work required but upon the speed with which it is to be done. It may be remembered also that the cardia with its weak, shallow but rapid contractions can dominate the rhythm of the antrum with its powerful, deep but slow contractions.

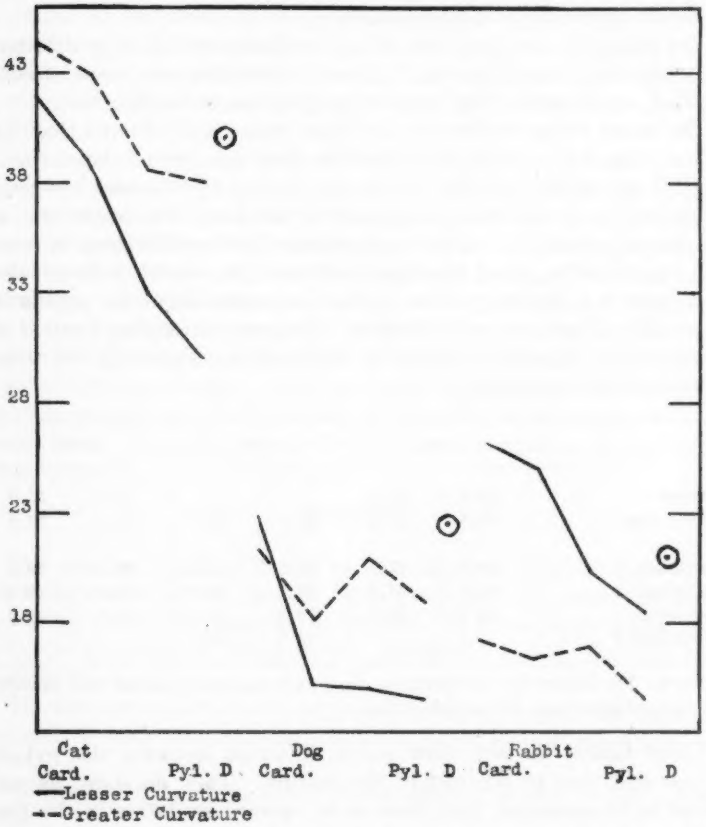

Fig. 1. Ordinates represent amounts of catalase. The three sets of abscissae represent segments of the stomach. The circle and dot represent the catalase content of the duodenal muscle, next to the pylorus. The solid line represents data from segments along the lesser curvature; the broken, from the greater curvature.

If we are correct in assuming that the catalase content of the muscle is an index of its metabolic activity, we may say that there is a metabolic gradient in the stomach similar to that in the intestine. This gradient probably underlies and accounts for the gradients of rhythmicity, irritability and latent period which we believe determine the direction of peristalsis in the stomach.

The muscle in the duodenum adjoining the stomach is very different in its rhythmic activities, latent period, irritability and shape of contraction curve and its high catalase content was to be expected.

The latent period studies on the intact stomach (2) showed that the pyloric ring was a little more irritable than the rest of the antrum. Gaskell has suggested that the muscle in the pyloric and ileo-cecal sphincters has a different phylogenetic origin from that in the rest of the digestive tract (4). This might account for the differences in reaction to adrenalin noted by some observers (5). Keith believes also that there is a rhythmic center in the duodenum about the papilla of Vater (6). It seemed well, therefore, to study the catalase content of a number of adjacent segments in this region. Following are some representative protocols:

	CAT		DOG		RABBIT	
Antrum	23.0	23.8			10.2	10.0
Pyloric ring	20.3	21.0	21.5	20.3	11.7	12.4
Duodenum 1	26.3	27.8	19.2	18.5	38.5	29.0
Duodenum 2	25.6	31.0	20.7	18.6	54.4	49.3
Duodenum 3	32.3	35.2	18.3	21.2	42.8	40.7
Duodenum 4			19.8	19.3		

NOTE: The figures for duodenum in the rabbit represent mucosa and muscle. The other figures are for muscle alone.

These figures do not show much difference between the pyloric muscle and that in the rest of the antrum. They do show, as was rather to be expected, that there is an upward gradation in the first few centimeters of the duodenum before the downward gradation to the ileum begins. Radiologists know that the so-called duodenal cap shows little activity in man and tends to remain filled during digestion. Alvarez has shown also in rabbits that muscle excised from this region has a comparatively poor rhythmicity (7). It appears, then, that the upper end of the intestinal gradient is not at the pylorus but a little

below, perhaps about the papilla of Vater. Although Keith's suspicion that there is nodal tissue in this region may be correct, it must be kept in mind clearly that one segment of intestine does not seem to influence the rhythm of the one next to it; the bowel does not pulsate like a heart and the word "pacemaker" must be used with caution. It is a little different in the stomach where the peristaltic rates of the different regions are all subordinated to that of the cardia. It is possible that some of the ripples which run down the intestine arise in the duodenum but records obtained so far suggest that they come over from the stomach.

We hope to take up, in a subsequent paper, the upsets in these gradients found in sickly animals. In all the work on gradients, both in the stomach and in the small intestine, it has been found necessary to make careful note of the condition of the animals as regards friskiness, nutrition, presence of snuffles, distemper, mange, intestinal parasites, etc. The gradients seem to be upset easiest and most frequently in the dog and least frequently in the rabbit. The inability of the rabbit to vomit may be due at least in part to the stability of its gastric and intestinal gradients. An even, steep downward gradient is probably more essential to the welfare of an herbivorous animal, with its rough bulky food, than to a carnivorous animal with its more fluid intestinal contents.

SUMMARY

The catalase content of the gastric muscle is graded downwards from the cardia to the pylorus. This gradient is more marked along the lesser curvature than on the greater. It corresponds pretty well with the gradients of latent period, irritability and rhythmicity previously observed.

The amount of catalase in the muscle seems to depend less upon the amount of work required than upon the speed with which it is to be done.

No peculiarity could be found in the catalase content of the pyloric ring.

Below the pylorus, the catalase content rises rapidly to a point in the middle or lower duodenum from whence the gradient is downward to the colon.

It is believed that these gradients of catalase content represent gradients of metabolism underlying and giving rise to the gradients of

rhythmicity and latent period which direct the downward waves of peristalsis.

These gradients are often reversed in sick animals which are vomiting or refusing food.

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XV. THE CATALASE CONTENT OF THE MUCOUS MEMBRANE FROM DIFFERENT PARTS OF THE DIGESTIVE TRACT

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Received for publication July 9, 1918

As is now well known, catalase is the ferment which liberates oxygen from hydrogen peroxid. It seems to have something to do with the oxidative processes and a good many writers feel that the catalase content of a tissue can be taken as a fair index of its metabolic activity. We have shown recently that the catalase content of the intestinal muscle is graded from duodenum to colon, i.e., a given weight of duodenal muscle has more catalase than the same weight of ileal or colonic muscle (1). When charted on coördinate paper, the gradients of intestinal rhythmicity, CO_2 production and catalase content were found to parallel one another very closely. There was a definite relation between the catalase content of any one bit of muscle and the degree of activity ordinarily displayed by the segment of intestine from which it was taken.

It seemed worth while, then, to see if we could demonstrate a similar relation between the catalase content of any one piece of mucous membrane and the activity (secretory and absorptive) of that part of the digestive tract. As we will point out later in the discussion, there are marked differences in the amounts of work done by the mucous membrane in different parts of the stomach and bowel. We shall show also that there are differences in the CO_2 production of equal weights of mucous membrane from different parts of the small intestine and colon.

TECHNIC

The technic has been described fully in a previous paper (1). The animals were killed either by a blow on the head or by chloroform and then bled. In dogs and cats the mucosa was peeled off from the muscle; in rabbits it was scraped off. The tissue was ground in a

Stomach—Lesser curvature

							Average
Cat:							
1. Near cardia	37.7	52.7	50.0	46.8	44.9	48.0	46.7
2.	37.4	30.0	50.7	46.2	29.5	31.2	37.5
3.	34.6	26.4	24.5	33.0	29.5	35.9	30.7
4. Near pylorus	32.3	26.1	21.0	30.1	24.4	27.0	26.8
Dog:							
1. Near cardia	21.8	27.4	36.8	34.4			30.1
2.	21.7	23.8	23.4	28.2			24.3
3.	20.8	18.8	20.1	23.6			23.8
4. Near pylorus	8.2	19.2	19.7	21.6			17.2
Rabbit:							
1. Near cardia	48.3	46.3					47.3
2.	41.4	35.3					38.4
3.	38.0	35.2					36.6
4. Near pylorus	35.9	28.8					32.4

Stomach—Greater curvature, upper duodenum

Cat:								
1. Near cardia	30.2	50.8	40.2	42.3	31.7	47.3	44.8	41.0
2.	48.0	36.6	40.0	35.4	33.2	40.5	33.5	38.2
3.	39.3	37.4	32.7	39.7	27.9	31.8	38.2	35.3
4. Near pylorus	29.6	33.5	31.0	40.4	17.4	27.7	26.1	29.4
5. Duodenum, near pylorus	41.4	56.3	58.0	55.6	26.2	32.5	38.6	44.1
Dog:								
1. Near cardia	13.5	18.8	36.3	31.0				24.9
2.	20.8	27.5	33.0	30.7				28.0
3.	24.2	21.6	23.6	25.1				23.6
4. Near pylorus	15.7	20.6	20.3	22.8				19.9
5. Duodenum, near pylorus	32.0	43.5	45.8	36.8				39.5
Rabbit:								
1. Near cardia	43.0	38.2						40.6
2.	38.7	26.0						32.4
3.	18.9	20.8						19.9
4. Near pylorus	21.8	20.2						21.0
5. Duodenum	31.9	29.9						30.9

Intestine

									Average
Cat:									
1. Duodenum	67.2	58.5	84.0	67.0	66.0	62.1	38.6	56.9	67.5
2. Jejunum	56.6	54.7	54.0	49.3	42.1	68.1	24.9	57.2	50.9
3. Middle	51.0	49.3	44.3	43.5	30.7	37.1	33.1	58.2	43.4
4. Ileum	51.8	48.3	35.9	43.2	27.1	40.1	29.4	36.8	39.0
5. Colon	33.7	30.0	37.0	35.0	30.2	34.2	23.9	28.0	31.5
Dog:									
1. Duodenum	44.8	49.1	51.0	46.5	49.6	40.2			46.9
2. Jejunum	32.4	39.4	52.0	47.2	32.4	31.3			39.1
3. Middle	30.7	32.2	45.7	34.2	35.1	30.0			34.7
4. Ileum	32.0	32.5	35.2	35.4	29.3	20.7			30.9
5. Colon	18.2	26.7	28.4	21.4	25.0	15.7			22.6

Colon

Cat:												
1. Upper	45.3	49.4	43.1	46.5	37.6	42.9	52.8	43.7	62.0	45.6	44.2	49.8
2.	50.0	37.4	28.3	39.6	42.5	26.1	48.0	42.5	44.7	59.8	40.7	41.8
3.	38.7	36.2	27.3	35.5	22.6	27.4	39.7	30.5	40.7	48.2	36.5	34.8
4.	24.6	33.9	24.9	34.6	21.8	26.7	37.1	30.7	29.0	38.1	40.3	32.9
5. Lower	27.0	23.5	40.7	31.1	35.0	29.2	58.3	51.4	30.7	27.4	31.5	35.1
Dog:												
1. Upper	23.3	36.2	36.6	42.4	34.9	25.9	26.0					32.2
2.	18.5	18.8	23.3	35.4	30.0	22.3	27.5					25.1
3.	18.6	21.5	30.0	22.3	26.8	21.2	24.9					23.6
4.	19.7	29.9	32.7	27.7	19.3	16.3	22.2					23.9
5. Lower	23.5	15.8	32.6	27.8	29.3	14.4	37.2					25.8

Cecum and Colon

Rabbit.					
1. Tip	42.0	45.4	20.6	32.4	35.1
2.	51.6	50.5	27.3	32.5	40.5
3.	49.8	54.6	29.2	26.8	40.0
4. Base	39.2	34.0	27.0	28.4	32.2
5. Colon	30.6	32.1	22.1	23.6	27.1

mortar and washed into a large test tube. To this were added 15 cc. of a 3 per cent H_2O_2 which had previously been neutralized. The oxygen which was liberated was measured in a burette at atmospheric pressure. By using a shaking machine five such tests on tissue from the same stomach or bowel could be run at the same time and under identical conditions. In the preceeding tables the figures represent

cubic centimeters of oxygen. These figures have not been reduced to a common temperature and atmospheric pressure because we are interested only in the gradations observed in the different sets of five readings. In all this work 0.2 gram of mucous membrane was used.

DISCUSSION

A glance at the tables and at figure 1 will show that definite differences in catalase content have been found: differences which are graded very much as they are with the muscle. Incidentally it should be noted that per unit of weight the mucous membrane contains about twice as much catalase as does the muscle. This is what we should expect from the work of Brodie and Vogt (2), who found the oxygen intake and CO_2 output of the mucosa much higher than that of the intestine as a whole.

Let us turn first to an analysis of the differences found in the stomach. It should be remembered that the stomach phylogenetically and embryologically is developed as a bulging in the primitive intestinal tube (3). Originally the dilated lower end of the esophagus joined the pyloric antrum at the incisura angularis. The pyloric antrum is the least modified portion of the primitive tube. The remnant of the esophagus along the lesser curvature forms the gastric canal, a furrow which serves to conduct fluids from the cardia to the antrum. As we should expect, the cells and glands in the mucous membrane in the pyloric antrum, around the cardia and along the lesser curvature, are least differentiated from those in the intestine (4). The acid-forming cells, peculiar to the stomach, are found in the body and fundus of the organ: regions which have developed as a cecum off of the original tube (5). It should be remembered also that the mucosa of the pyloric antrum probably has little function. The antrum serves as a muscular gizzard in which the food is ground up and mixed with juices which trickle down from above.

On turning to the table we find the figures a little larger on the greater curvature than on the lesser. That is what we should expect as the functions of the gastric canal probably do not require great metabolic activity. As we should expect also, there is very little catalase in the mucosa of the pyloric antrum. It is hard to say, however, why there should be so much in the cells around the cardia. One would not expect much digestive activity in that comparatively quiet part of the stomach. It is interesting that the amounts of catalase in

the stomach are considerably lower than those in the upper small intestine. This is what we should expect, as the small bowel is the essential organ of d gestion. The stomach serves largely as a hopper to pass the food downward in small amounts as needed. A glance at figure 1 will show that the catalase content of a given weight of the gastric mucosa is even less than that of the same weight of colonic

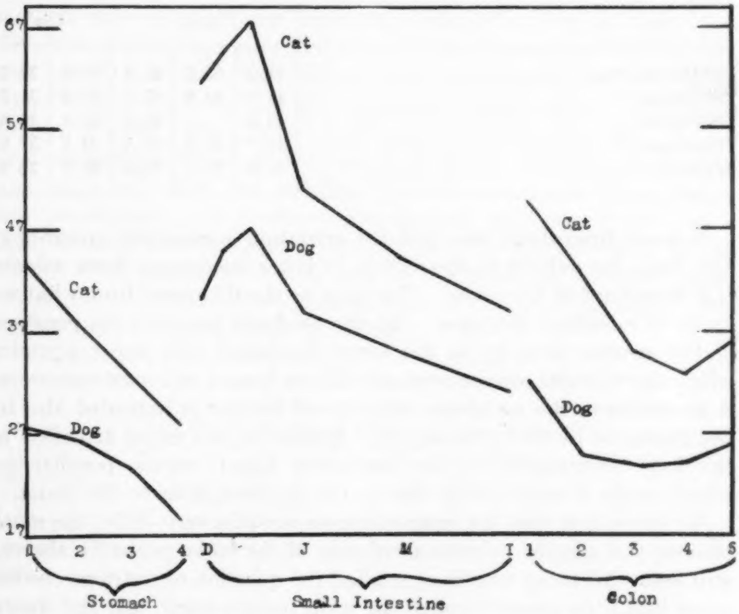

Fig. 1. Shows the gradation in catalase content of the mucosa in different regions. The abscissae represent the regions and the ordinates cubic centimeters of oxygen liberated in fifteen minutes by 0.2 gram of tissue.

mucosa. It should be noted, however, that the gastric mucosa is thick and that in the colon very thin so that the metabolic activity of a given area of the gastric lining is probably much greater than that of the same area in the colon.

On turning to the small intestine it is apparent that there is an abrupt change at the pylorus from a mucous membrane poor in catalase to one rich in catalase. When the data were averaged we found that

the duodenal figure in the "greater curvature" tables was greater than that in the "small intestine" table. This seemed to be due to the fact that in the first experiments we had used mucosa next to the pylorus; in the later ones it had been taken from farther down the duodenum. To make sure that the differences were not accidental, the catalase was measured in several adjacent segments as follows:

	CAT			DOG	
Pyloric antrum	34.0	35.8	32.8	17.6	23.3
Duodenum 1	41.5	43.5	37.9	37.5	31.7
Duodenum 2	43.3		38.5	41.1	35.8
Duodenum 3	45.7	45.6	39.5	32.5	37.8
Jejunum	41.6	36.7	36.6	37.7	36.9

It seems from these data that the gradation is generally upwards at first from the pylorus to the middle or lower duodenum, from whence it is downward to the colon. This is as we should expect from what we know of intestinal digestion. All the evidence points to the presence of the greatest activity in the lower duodenum and upper jejunum where the valvulae conniventes and villi are largest and most numerous. A gradation in the metabolic activities of the gut is indicated also by the gradation in the blood supply. Monks (6) has called attention to the local peculiarities of the mesenteric blood vessels, peculiarities which insure a larger blood flow to the jejunum than to the ileum.

We know now that the gastric mucosa absorbs very little; the much thinner and simpler mucous membrane of the colon probably absorbs still less. All those who have studied the question of nutrient enemas agree that little passes through the walls besides sugar, salt and water. This absorption of water seems to be one of the main functions of the colon. In fact, this organ is to the body what a condenser is to a steam engine. There are few glands in the colonic wall and these are concerned mainly with the excretion of mucus. As we should expect, then, the catalase values are low. They are graded downwards from the cecum at first but later they rise toward the anus. It is impossible to say at present just why this last rise should appear.

The cecum in the rabbit plays a more important part in digestion than does the colon and we find its mucosa has a larger catalase content. As we would expect, there is no constant gradation in the figures from different parts of the cecum. Most of the work is probably done

by the cells in the middle region of the organ where the bulk of the contents is greatest, and there the catalase content is highest.

On the whole, these results are compatible with the theory that the catalase content of a tissue is an index to its metabolic activity. In order to establish this point a little more definitely, a few estimations were made of the CO_2 production of weighed amounts of mucous membrane taken from five different regions of the cat's bowel. Haas' method was used. It has been described in a previous paper (1). The bits of tissue are sealed into small tubes containing Locke's solution which is stained pink with a trace of phenolsulphonephthalein. The CO_2 given off acidifies and decolorizes the solution. After some time in a water bath, the tubes are graded according to tint. As the mucous membrane does not become active in the Locke's solution the way the muscle does, the decolorization was slower and the gradation less pronounced. In fourteen experiments, however, there was a definite downward tendency from the duodeno-jejunal region to the colon. This gradation is seen also in the following six experiments where the faded tubes were titrated back to their original color. The figures represent drops of n/50 NaOH.

	TIME						
	3 hours	2½ hours	5¾ hours	3½ hours	3½ hours	3½ hours	Average
Duodenum........	10	9	7	10	9	11	9.3
Jejunum..........	10	12	11	13	15	14	12.5
Middle............	8	9	6	10	6	12	8.5
Ileum.............	6	10	6	9	9	9	8.1
Colon.............	8	8	7	8	8	9	8.0

Although the data are few and somewhat irregular in their gradation, they tend strongly to confirm the conclusions arrived at from the catalase studies.

Do these studies throw any light on the predilection of ulcer for certain regions? We believe they do not. According to Burge and others (7), a loss of oxidative power in the mucous membrane will lead to autodigestion by the contained ferments. Magath (8) believes that lumbricoid worms in the intestine owe some of their immunity to trypsin to the presence of considerable amounts of catalase in their integuments. Our studies lend little support to these views because, if conditions in man are similar to those in the cat and dog, the favorite

site for ulcer, in the upper duodenum, must have a very high catalase content. The low catalase content of the mucous membrane in the antrum might help the formation of ulcers there but we know that they often are found well up on the lesser curvature where the catalase content should be high.

It is very suggestive that the commonest site for cancer in the digestive tract, the lesser curvature near the pylorus, should have the lowest catalase content. The next lowest place in the animals studied would correspond in man to about the splenic flexure, another common site for cancer. Child (9) has shown with simple forms of life that the progressive differentiation of the cell, with the gradual accumulation of more or less stable structural substances, leads to senescence and death. If the rate of metabolism be greatly slowed by adverse circumstances, such as starvation and drying, these old cells may use up their metaplasm; they dedifferentiate, and in so doing reacquire the faculty of growth and multiplication. Goodpasture (10) has just pointed out how this mechanism, so helpful for the rejuvenation of lower forms of life, can become a menace to the existence of higher forms. It may be, then, that these regions of the mucous membrane with their low rates of metabolism are most senile and therefore most susceptible to cancerous growth. We now have some explanation also for the remarkable immunity of the duodenal mucosa not only to primary cancer but to invasion by growths beginning in the stomach. Pathologists have often wondered why these gastric tumors are always so sharply limited at the pylorus. A glance at figure 1 shows now, as might have been expected, that there are pronounced differences in the metabolism of the cells on the two sides of the sphincter: differences which can, conceivedly, favor or hinder the growth of cancer.

SUMMARY

There is considerable evidence for the view that the catalase content of a tissue is an index of its metabolic activity.

In the gastric mucosa the catalase content is graded downwards from the cardia to the pylorus. The amounts are smaller along the lesser curvature than along the greater. In the small intestine the amounts are graded upwards at first to the middle duodenum and then downwards to the ileo-cecal sphincter.

There is comparatively little catalase in the colonic mucosa and that is graded downwards in the first two-thirds of the tube. The low

metabolic activity in this region is against the idea that colonic auto-intoxication is a common happening. The mucosa of the rabbit's cecum is quite rich in catalase. It shows no constant gradation from one end to the other.

The CO_2 production of equal weights of mucosa is graded from the duodeno-jejunal region to the colon.

These graded results agree quite well with what we know about the digestive activities in the different regions. They do not appear to throw any light on the predilection of ulcer for certain locations.

It is suggestive that the commonest site for cancer has the lowest catalase content. This region with its low rate of metabolism is perhaps most senile and thus most disposed to malignant change.

The abrupt change at the pylorus to a mucous membrane with a high rate of metabolism may easily account for the sharp limitation of gastric cancer usually found at that point.

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A COMPARISON OF THE ACTIVITY OF EXTRACTS OF THE PARS TUBERALIS WITH EXTRACTS OF OTHER REGIONS OF THE OX PITUITARY

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Received for publication July 12, 1918

INTRODUCTION

For some time it has been recognized that the epithelial portion of the hypophysis cerebri consists of two very different parts. One is bulky and glandular while the other is a thin, epithelial layer closely investing the neural lobe. The two parts are separated by a cleft which is the residuum of the original cavity of Rathke's pocket. To the former part Peremeschko (1) has applied the name "Korkschicht" and to the latter "Markschicht," while Herring has termed them "anterior lobe proper" and "pars intermedia" respectively.

Only recently has it been shown that a distinct third epithelial lobe exists, extending nasalwards from the junction of pars intermedia and anterior lobe proper, to surround the infundibulum and to spread out for a variable distance under the brain floor. Because of its close relation to the tuber cinereum in many animals Tilney (2) has named this part the "pars tuberalis."

The pars tuberalis had long been considered as belonging to the pars intermedia. In fact Herring (3) calls it the "tonguelike process of the pars intermedia" and the "extension of pars intermedia round neck of pituitary," although he notes that it is more vascular than that part of the pars intermedia which faces the cleft.

Tilney has shown that the pars tuberalis is distinct histologically and embryologically. One of us—Atwell (4)—has recently given a full account of the development of the pars tuberalis in the rabbit. It is shown to arise from a pair of thickened ridges which are situated at the nasal side of Rathke's pocket and which—contrary to the statements of Tilney—are discernible very early. The two ridges develop into the lateral lobes which later come to lie spread out under the brain

floor and which, fusing with each other across the midline, form the pars tuberalis of the adult.

The pars tuberalis has been recognized and its development traced in several vertebrates. Tilney (2) gives a brief account of its development in the cat and the chick; Baumgartner (5) has studied its developmental history in the reptilia; Parker (6) in the marsupialia; and Atwell (4) in the rabbit and (7) in the tailless amphibia. It is well known that the ventral sacs of the hypophysis of certain elasmobranchs have a paired origin and Woerdemann (8) has homologized these sacs with the "lobulus bifurcatus" (pars tuberalis) of the higher vertebrates. The history of the pars tuberalis in the ganoids and the teleosts is practically unknown but it is significant that three different epithelial portions have been recognized. Thus Stendell (9) speaks of a "Hauptlappen," a "Zwischenlappen" and an "Uebergangsteil." Whether the last-named portion is homologous with the pars tuberalis of other vertebrates and whether it arises from a pair of anlagen in these fishes remain to be learned.

The constancy with which the pars tuberalis is present in various vertebrates bespeaks for it, the senior author was led to believe, some distinct physiological function. Accordingly the present study was undertaken with a view to determining what functions, if any, are possessed by it. Since the pars tuberalis has been most often confused in structure with the pars intermedia, our first endeavor has been to compare the activity of extracts prepared from these two parts. This study will not include a comparison of the functions of the pars tuberalis and the anterior lobe proper. Further experimentation along this line is projected.

THE ANATOMY OF THE BOVINE HYPOPHYSIS

In our work we have made use of the pituitary body of the ox, since it is large and easily obtainable. Because the topography of the pars tuberalis has never been described for the hypophysis of the ox, our physiological experiments were necessarily preceded by a rather careful morphological study of the gland. It is extremely important to recognize the extent and relations of the several parts of the organ when "pure" extracts are to be made. We feel that the finer anatomy of the hypophysis too often has been neglected by physiological workers.

The pituitary body as ordinarily received from the packing house has been torn loose from the brain leaving a longer or shorter stalk. Such

glands are unfavorable for microscopical study since not all of the pars tuberalis is present. By carefully removing the hypophysis with a considerable portion of the diencephalon and hypothalamus attached for fixation one is able to preserve the attachment of the neural stalk to the infundibulum together with the entire pars tuberalis.

Figure 1 presents a diagrammatic mid-sagittal section through the hypophysis and the adjacent brain floor. The anterior lobe proper (fine stipple) makes up the main bulk of the gland. The pars intermedia (black) is a thin epithelial layer closely applied to the neural lobe. Pars intermedia and anterior lobe proper are separated by the cleft or residual lumen of Rathke's pocket. Near the caudal end of the pars intermedia

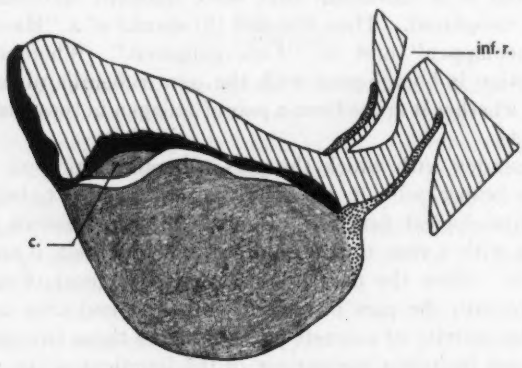

Fig. 1. Diagrammatic median sagittal section of the bovine hypophysis, nasal end at the right. × 4. Lined, neural lobe, neural stalk and brain wall; black, pars intermedia; fine stipple, pars anterior propria; coarse stipple, pars tuberalis; *inf. r.*, infundibular recess; *c.*, cone of Wulzen.

is a mass of cells resembling the anterior lobe in structure (*c*, fig. 1). This is the cone of Wulzen (10). We are able to confirm the reports of this observer as to the constancy of its presence. In some glands (as shown in our fig. 1) the cone is flattened, in others it is much taller. It is characterized by the presence of numerous acidophile cells such as are so abundant in the anterior lobe proper.

The neural lobe (lined, fig. 1) is attached to the brain floor by a narrow stalk into which the infundibular recess (*inf. r.*) extends for some distance. It is surrounding this neural stalk that the pars tuberalis (coarse stipple, fig. 1) is to be found. It forms a thin layer varying from 0.25 mm. to 1.5 mm. in thickness. While the boundary between

the pars tuberalis and the pars intermedia is fairly well defined, the same cannot be said for the junction of the pars tuberalis with the anterior lobe proper. In fact, the pars tuberalis seems to extend for a variable distance over the anterior lobe and to form a cortex for its nasal end.

Histologically the pars tuberalis is readily distinguished from both the pars intermedia and anterior lobe proper. It does not contain the eosinophile cells which are so characteristic of the anterior lobe and it is much more vascular than the pars intermedia. Even in the gross numerous blood vessels may be discerned, disposed in an antero-posterior direction. These are well shown in transverse section in figure 2. The cells of the pars tuberalis are arranged in small blind tubules or acini, the walls of which are composed, generally, of one layer of cells (fig. 3). Occasionally the lumen of an acinus is slightly distended by an accumulation of homogeneous material which takes a faint eosin stain. Many acini do not show a distinct lumen. The cells of the pars tuberalis are lighter than those of the pars intermedia although both have been described as "chromophobe."

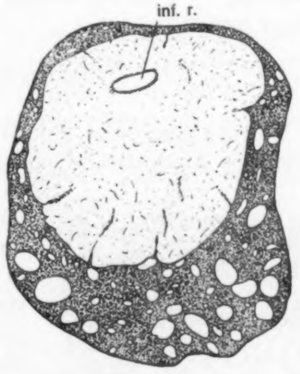

Fig. 2. Transverse section of the hypophyseal stalk. × 20. Neural stalk (light) is in center and is surrounded by the pars tuberalis which shows numerous blood vessels; *inf. r.*, infundibular recess.

Some difference of opinion seems to exist concerning the presence of hyalin bodies in the neural lobe. Lewis, Miller and Matthews (11) state that such bodies do not form a striking picture in the histology of the ox hypophysis. Herring (12) on the other hand finds them very pronounced, some showing the remains of swollen nuclei; "others are in a more advanced stage of alteration and form rounded, oval or irregular colloid masses." We must agree with Herring that hyalin bodies are fairly numerous in the tissues of the neural lobe and that they are also to be found, though less abundantly, in the neural stalk. After routine methods of preparation (formalin fixation, hematoxylin and eosin staining) the hyalin bodies show a faint eosin color and might be very easily overlooked. After a modified Weigert hematoxylin and van Gieson connective tissue stain, however, they are a very dark gray and stand out prominently.

They often contain in their centers darker staining masses. Whether or not these are degenerating nuclei, as claimed by Herring, we are not prepared to state.

The hyalin bodies found in the neural lobe are different chemically from the contents of the acini of the pars tuberalis. This is clearly shown in the Weigert-van Gieson stain. While the hyalin masses stain a dark gray color the material in the acini is colored a light yellow. It seems most likely that the hyalin bodies are derived from the pars intermedia and enter the neural lobe secondarily, as maintained by Herring. We do not believe that the pars tuberalis takes any part in their formation.

THE PREPARATION OF EXTRACTS

For the purpose of comparing the activity of the pars tuberalis and the pars intermedia we endeavored to prepare "pure" extracts which would contain the active principle or principles of each part separately. By using the methods of Lewis, Miller and Matthews (11) and of Herring (12), it was found a comparatively easy matter to obtain the pars intermedia substance in the pure condition. Upon separating the anterior lobe proper from the "posterior lobe" (which consists of neural lobe and the closely investing pars intermedia), by cutting around the margins of the cleft, the surface of the pars intermedia is exposed. Its color is usually distinctive ranging through almost all shades of yellow, orange and brown. There is no marked intermingling of nervous and pars intermedia tissues in the ox (cf. fig. 1). By using a pair of small curved scissors and working where the pars intermedia is the thickest (near the caudal end of the gland) it is possible to snip off small pieces of the yellowish tissue without cutting deep enough to include any of the neural lobe, which is gray. Care was taken to avoid the margins of the cleft and thus we are sure that we prevented contamination from the anterior lobe proper or from the pars tuberalis. The cone of Wulzen was readily recognized and was always avoided.

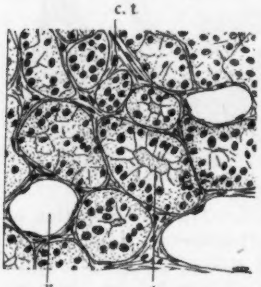

Fig. 3. Section of pars tuberalis of bovine hypophysis. × 300. *l.*, lumen of an acinus distended by a small amount of granular material; *c.t.*, connective tissue; *v.*, one of the smaller vessels.

The separation of the pars tuberalis from the neural stalk which it invests has been supposed to be impossible. For example Herring (12) states, in reference to his "tongue-like process of the pars intermedia" and the neural stalk, "it would be impossible to dissociate the two tissues at this level." After a careful study of the physical properties of the two tissues, however, we have found that they may be separated very satisfactorily. The pars tuberalis is well supported by connective tissue which ramifies throughout its substance (fig. 3). On this account it is tough and not readily broken. The tissue of the neural stalk, on the other hand, is soft and friable.

After some experimenting it was found that the most satisfactory procedure was as follows: the stalk of the gland (the neural stalk and the surrounding pars tuberalis) was cut transversely a sufficient distance anterior to its attachment to the gland to avoid the pars intermedia and the anterior lobe proper. The short cylinder was laid upon a clean glass plate and the pars tuberalis slit its length with fine scissors. It was then found to be a comparatively easy matter to peel off the pars tuberalis as a thin, tough membrane. Any adhering pieces of nervous tissue were removed by gentle scraping with a blunt scalpel. Both the pars tuberalis and the neural stalk tissues were saved.

For the preparation of extracts, then, we had pure pars intermedia, pure tissue of the neural stalk, and pars tuberalis which had, perhaps, a small amount of neural stalk substance adhering but which certainly was uncontaminated by inclusion of any of the pars intermedia or of the anterior lobe proper.

From these tissues we at first prepared extracts by macerating the fresh gland substance in a mortar and then boiling it with dilute acetic acid as described by Roth (13) so that each cubic centimeter of the completed extract represents the water-soluble constituents from 1 mgm. of fresh gland substance. We found this dilution entirely too weak for most of our experiments so that later we prepared extracts having a strength of 10 per cent of the fresh gland. Each cubic centimeter of the stronger extract represents the water-soluble constituents from 100 mgm. of fresh gland substance. Dilutions of this extract were made as required. In certain instances comparisons were made between the action of these extracts and of commercial pituitrin and adrenalin.

THE ACTION OF EXTRACTS

Since the discovery in 1895 by Oliver and Schäfer (14) that extracts of the pituitary body contain an active physiological substance, a number of studies have been made attempting to determine the origin of this substance. Howell (15) soon found that the active principles are resident in the posterior lobe and not in the anterior, an observation which has been confirmed repeatedly. In 1908 Herring (3) showed from histological evidence that the "posterior lobe owes its activity to the investing epithelial layer, the pars intermedia." Crowe, Cushing and Homans (16) concur with Herring in stating that hyalin bodies pass into the third ventricle. Cushing and Goetsch (17) obtained a pressor effect by injecting concentrated human cerebro-spinal fluid into rabbits. Carlson and Martin (18), however, were not able to obtain a pressor effect from the cerebro-spinal fluid of normal dogs.

Lewis, Miller and Matthews (11) made extensive studies upon the activity of extracts from various parts of the ox pituitary. From extracts of pure pars intermedia they obtained a pressor effect on dogs. These authors tested extracts made from the stalk of the gland or "infundibular walls" and failed to obtain pressor effects. On this account they doubt the passage of secretion from the neural lobe into the third ventricle. Biedl was able to confirm the observation that a pressor substance is yielded by extracts of the pure pars intermedia, but he failed to obtain constantly such effects from the neural lobe. Herring found that the pars intermedia of the ox pituitary yields a substance which stimulates uterine contraction and increases the secretion of milk but which exercises little if any pressor effect in the cat. Extracts of the neural lobe were found "to yield, in relatively larger amount, the material which stimulates the uterus and acts as a galactagogue." In addition, extracts of the neural lobe produce a rise of blood pressure in the cat.

The use of pituitary extracts to stimulate peristalsis in cases of postoperative intestinal stasis has become quite general since its suggestion by Bell and Hick (19). Duffy (20) has recently reviewed the literature and strongly recommends pituitary extract for the relief of intestinal paresis. Certain authors, however, have noted that inhibition of the normal intestinal rhythm may be produced by pituitary extracts. Bayer and Peter (21) using 0.4 to 5.0 cc. of pituitrin in 15 to 20 cc. Ringer's solution obtained marked slowing and inhibition of the rhythm of segments of rabbit intestine. Shamoff (22) used a much greater

dilution and obtained inhibition of activity on the isolated intestinal segment. When 1 mgm. of dried posterior lobe substance was shaken up in 5 cc. of Ringer's solution and later the clear fluid decanted and added to the Ringer's solution containing the intestinal segment, a distinct inhibitory effect was observed. A similar effect was obtained with certain commercial pituitary extracts in a dilution of 1:5000. Hoskins (23) observed inhibition of peristalsis in the intact intestine of the dog following intravenous injection of pituitrin, and also following injection of saline extracts prepared from the desiccated gland. Roth (13) obtained inhibition of the intestinal segment of the rabbit in pituitrin diluted 1:40. He ascribes the inhibitory effect to the presence of chlorbutanol, which is used as a preservative in this pituitary extract.

Our experiments include tests made to determine what action, if any, is possessed by the pars tuberalis of the ox pituitary upon the isolated uterine segment, the isolated intestinal segment, and what action upon the blood pressure of the rabbit and of the dog.

a. Upon the isolated uterine segment. The effect of extracts of the pars tuberalis and of the pars intermedia was tested upon the isolated uterine segment of young guinea pigs. The method described by Dale and Laidlaw (24) was followed. The uterus of a virgin guinea pig weighing 300 to 350 grams was employed. It was suspended in about 100 cc. of oxygenated Locke's solution and maintained at 38 to 39°C. Measured quantities of the extracts to be tested were added to the Locke's solution and allowed to act until a maximum contraction had been obtained. This was ordinarily not longer than thirty seconds. After washing with fresh Ringer's at least five minutes was allowed before another test was attempted.

The extracts used were the weaker (Roth's "standard"), each cubic centimeter representing the water-soluble principles from 1 mgm. of the fresh gland. In a preliminary test 2 cc. of pars intermedia extract elicited a contraction of 35 mm., while 2 cc. of pars tuberalis extract gave a contraction of only 5 mm. The record of one series of readings obtained consecutively is given in table 1, page 84.

Computing from this table, the average contraction value in millimeters for each cubic centimeter of pars intermedia extract is 33; while the same average value per cubic centimeter of pars tuberalis extract is 4.45 mm. Thus we find that extracts of the pars tuberalis are very much inferior to extracts of the pars intermedia in producing contraction of the isolated uterus of the guinea pig. By computing the rela-

tive activity of the two extracts it will be seen that the pars intermedia extract is approximately seven times as powerful as the pars tuberalis extract.

b. Upon the isolated intestinal segment. The several pituitary extracts were tested for their effects upon the isolated intestinal segment of the rabbit. Commercial pituitrin was also tested in a number of dilutions. The rabbit was stunned by a blow, then bled, this procedure being chosen to avoid the inhibitory effect produced by an anesthetic. The intestinal segment was suspended in warm oxygenated Ringer's solution in the same apparatus used for the uterine segment experiments.

We have not observed inhibition of intestinal rhythm by the action of pituitary extracts except in rather weak dilutions. Comparatively strong extracts increased the activity of the segment. When 0.5 cc.

TABLE 1

Uterus test

TIME	EXTRACT TESTED 1:1000	HEIGHT OF RESULTING CONTRACTION	
		Pars intermedia	Pars tuberalis
		mm.	*mm.*
4:47	5 cc. pars tuberalis		20
4:53	5 cc. pars tuberalis		22
4:59	5 cc. pars tuberalis		20
5:05	1 cc. pars intermedia	34.0	
5:10	0.5 cc. pars intermedia	15.5	
5:15	5 cc. pars tuberalis		27

of 10 per cent extract of pars intermedia is added to the segment contracting spontaneously in 100 cc. Ringer's solution, a slowing of the rhythm and a strengthening of the contractions is produced. A similar effect was caused by the addition of 0.5 cc. of pituition, except that the effect was more marked.

Inhibition of intestinal activity was obtained in certain cases by the use of very dilute extracts. Like Shamoff, we find that the inhibitory effect is not a constant phenomenon. On one occasion a clear-cut case of inhibition was obtained by shaking 1 mgm. of dried pars intermedia with 5 cc. of Ringer's solution and adding the supernatant fluid to the test bath. The similar use of 1 mgm. of dried pars tuberalis failed to produce an inhibitory effect.

In figure 4 is shown the effect produced by *A*, 0.2 cc. of 1:1000 adrenalin chloride; *B*, 5 cc. of Ringer's solution shaken up with 1 mgm. dry pars intermedia; and *C*, 5 cc. Ringer's shaken up with 1 mgm. dry pars tuberalis.

c. Upon the blood pressure. 1. Of the rabbit. A female rabbit weighing 3.6 kilos was anesthetized by paraldehyde *per os;* the right jugular

Fig. 4. To show the effect of various extracts upon the isolated intestinal segment of the rabbit contracting spontaneously in oxygenated Ringer's solution. Downstroke represents contraction.

vein was exposed for the administration of extracts and changes in blood pressure were recorded from the left carotid. The extracts tested in this experiment were of the weaker variety (1:1000 of the fresh gland). The intravenous injection of 1 cc., 2 cc. or 5 cc. of 1:1000 extract of pars tuberalis failed to call forth any pressor effect. The administration of 2 cc. of 1:1000 extract of pars intermedia, however, elicited a slight rise of pressure while 5 cc. of the same extract produced

a well-defined rise (fig. 5). The pressor effect was of comparatively short duration.

We find, then, that an extract of the isolated pars intermedia of the ox pituitary has a distinct pressor effect upon the blood pressure of the rabbit when injected in amounts as small as the equivalent of 2 to 5 mgm. of the fresh gland substance. Equal amounts of the pars tuberalis fail to produce a rise in pressure.

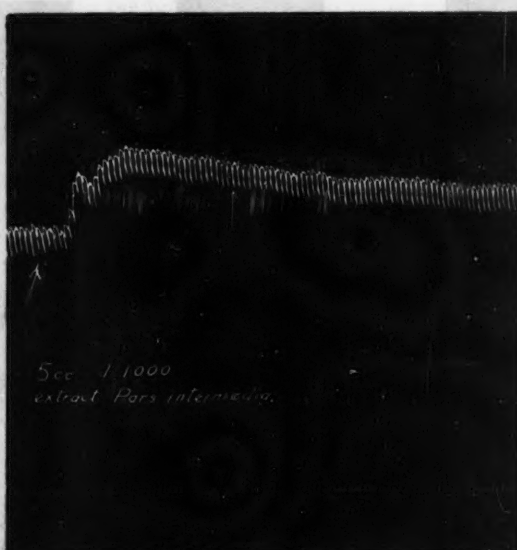

Fig. 5. To show the effect upon the blood pressure of the rabbit following intravenous injection of pure pars intermedia extract. Time is in seconds.

2. Of the dog. Intravenous injection of 0.3 cc. pituitrin made up to 5 cc. in salt solution produced a typical prolonged rise of pressure preceded by a brief depression. After about thirty minutes 0.6 cc. of 10 per cent extract of pars intermedia in 5 cc. salt solution called forth a strikingly similar reaction consisting of a preliminary depression and a subsequent prolonged rise.

In two experiments the effects of intravenous injections of commercial pituitrin, extract of pars tuberalis and extract of neural stalk were compared. The two experiments gave similar results and only the data from experiment 2 will be presented in detail.

A male dog weighing 20 kilos was anesthetized by intraperitoneal injection of chloretone in olive oil. The extracts were introduced into the left femoral vein and the blood pressure record was obtained from the left carotid artery. The extracts of pars tuberalis and of neural

TABLE 2

Blood pressure in the dog

NO.	DOSE	EXTRACT	RISE OF BLOOD PRESSURE IN MILLIMETERS OF MERCURY
	cc.		mm.
A	2	Pars tuberalis	6.5
B	4	Pars tuberalis	14.5
C	2	Neural stalk	36.5
D	2	Pars tuberalis	6.5
E	1	Pituitrin $\frac{1}{10}$	16.0
F	1	Neural stalk	19.0
G	4	Pars tuberalis	12.0
H	1	Neural stalk	20.5
I	4	Pars tuberalis	12.0
J	1	Pituitrin $\frac{1}{10}$	18.0

Summary from table 2. Averages of blood pressure reactions (rise of blood pressure in millimeters)

PITUITRIN 1 CC. ($\frac{1}{10}$)	PARS TUBERALIS 2 cc.	PARS TUBERALIS 4 cc.	NEURAL STALK 1 cc.	NEURAL STALK 2 cc.
16.0	6.5	14.5	19.0	36.5
18.0	6.5	12.0	20.5	
		12.0		
Av. 17.0	6.5	12.8	19.7	36.5

Average pressor value in millimeters per cubic centimeter of extract

PITUITRIN $\frac{1}{10}$	PARS TUBERALIS	NEURAL STALK
17.0	3.2	19.0

stalk used in this experiment were equivalent to 10 per cent of the fresh gland; that is, each cubic centimeter of the extract represented the water-soluble constituents of 100 mgm. of fresh gland substance. The pituitrin used was diluted to one-tenth the strength of commercial pituitrin.

Computing from the above data it is seen that a 10 per cent extract of neural stalk is somewhat more powerful in raising blood pressure in the dog than is an equal amount of 10 per cent pituitrin. Extracts of the pars tuberalis are very inferior to those of the neural stalk in pressor effect. Numerically expressed, extracts of the neural stalk are almost six times as powerful as extracts of the pars tuberalis.

DISCUSSION

Our observations have shown that extracts of the pars tuberalis are very inferior to extracts of the pars intermedia in producing contraction of the isolated uterine segment and likewise are inferior to extracts of the neural stalk in raising the blood pressure of the dog. Two things especially are to be noted. First: the pressure curves produced by these three extracts are similar, indicating that we are dealing with one substance or at least with the same group of substances in all cases. Second: the method of preparation of the extracts makes it certain that the pars intermedia and neural stalk extracts were pure, but it is highly probable that the pars tuberalis extract was contaminated by the inclusion of small amounts of the neural stalk.

It is our belief that the active effects shown by extracts of the pars tuberalis are due to the inclusion of neural stalk substance. In other words we believe that if an extract of the pars tuberalis could be obtained in an absolutely pure condition it would not be capable of the active effects which we have noted.

Lewis, Miller and Matthews (11) failed to obtain a pressor effect from extracts of the "infundibular walls" and so concluded that there is a distinct break in the path of secretion between the neural lobe and the third ventricle. At the time these authors performed their experiments the existence of the pars tuberalis as a distinct lobe had not been recognized. It has been our observation that in glands received from the packing house there is considerable retraction of the soft neural tissue in the centre of the stalk, leaving a false "infundibulum" the walls of which are composed mainly of the pars tuberalis. This tissue is very feeble in producing pressor effects, as we have shown. In the preparation of our neural stalk extract we used only careful isolated neural tissue and have obtained constantly a striking pressor effect on the dog. It is believed that this observation removes the objection made by Lewis, Miller and Matthews against the possibility that secretion may pass from the neural lobe into the third ventricle *via* the neural stalk.

Biedl (25) states that extracts of pure neural tissue of the hypophysis do not produce a pressor effect, but Herring has controverted this statement since he has been able to obtain constantly a rise of pressure in cats with such extracts. Our observations on the effects produced by extracts of the isolated neural stalk lend support to the contentions of Herring.

Lewis, Miller and Matthews and likewise Biedl obtained a marked rise of blood pressure in the dog by intravenous injection of pure pars intermedia extracts. Herring, however, failed to obtain similar effects in the cat. We have obtained strong pressor effects in the dog, thus confirming the observations of Lewis, Miller and Matthews and of Biedl. Of course, as Herring suggests, the difference of results obtained may be due to differences in reaction peculiar to the species of animal used. We have not tested the effect of pars intermedia extract on the cat but we are able to state that in the rabbit an extract representing as little as 5 mgm. of fresh pars intermedia of the ox causes a distinct rise of pressure.

The depression or complete inhibition of intestinal activity which sometimes follows administration of pituitary extracts is as yet an imperfectly understood phenomenon. In reviewing the results of those few observers who have obtained such depression one is struck by the wide variance of concentration in the extracts used. Of authors who record depression with the isolated intestinal segment Bayer and Peter (21) and Roth (13) used comparatively concentrated solutions, while Shamoff (22) and ourselves have used very dilute solutions. Hoskins (23) alone has recorded inhibition of activity in the intact intestines.

Bayer and Peter obtained inhibition by the use of from 2 to 5 cc. pituitrin in 15 to 20 cc. Ringer's solution, and Roth observed inhibition when the intestine segment was suspended in pituitrin diluted 1:40. Roth ascribes the inhibitory effect to the chlorbutanol present in this pituitary extract since he obtained a similar inhibition with a solution containing an equivalent amount of chlorbutanol alone. This is probably the correct explanation when inhibition is obtained in such enormous concentrations. But this certainly does not apply to the inhibitions recorded by Shamoff who did not use ordinary pituitrin and who obtained only slight depression with "pituitrin A," while he obtained more distinct inhibition with other commercial preparations as well as with extracts prepared from fresh and dried glands.

The only inhibition of clinical interest is that resulting from the weaker dilutions since it is manifestly impossible to attain a concentra-

tion as great as 1:40 for instance, in the living animal. Further observations on the intact intestine are necessary to determine whether such inhibitory effects constitute an indication against the use of pituitary extracts for the relief of intestinal stasis.

SUMMARY AND CONCLUSIONS

1. A "pars tuberalis" may be distinguished in the bovine hypophysis. It differs histologically from both the pars intermedia and the pars anterior propria.

2. The pars tuberalis may be separated fairly definitely from the neural stalk which it invests and may be obtained in sufficient quantity for the preparation of extracts.

3. The extract of pars tuberalis so obtained is doubtless contaminated by the presence of small amounts of neural stalk substance but it is certain that there is no admixture of tissue from the pars intermedia or from the pars anterior propria.

4. Extracts of the pars tuberalis are very inferior to extracts of the pars intermedia in producing contractions of the isolated uterine segment and are likewise inferior to extracts of the neural stalk in raising the blood pressure of the dog. Extracts of the pars tuberalis equivalent to 5 mgm. of fresh gland substance failed to produce a rise of blood pressure in the rabbit.

5. It is believed that the active effects displayed by extracts of the pars tuberalis are due to an inclusion of some of the neural stalk substance during the preparation of the extract. It would seem improbable that the pars tuberalis is responsible for the origin of active principles which increase uterine contraction or raise the blood pressure.

6. An extract of the pure pars intermedia of the ox is capable of producing a strong pressor effect when injected intravenously into the dog. In this we confirm the observations of Lewis, Miller and Matthews and of Biedl. We are able to add that an extract of pure pars intermedia representing 5 mgm of the fresh gland substance produces a distinct rise of blood pressure in the rabbit.

7. An extract of the pure neural stalk is fairly powerful in producing a rise of blood pressure in the dog. This removes one objection to the possibility that the secretion of the pars intermedia passes into the neural lobe and then, *via* the neural stalk, into the third ventricle.

We wish to express our sincere thanks to Prof. C. W. Edmunds for the use of apparatus in the pharmacological laboratories and for very generous personal assistance in the animal experiments. We wish also to thank the research laboratory of Parke, Davis & Company for numerous courtesies shown us during the course of these studies.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

VIII. The Effects of Quinine on the Production of Egg Yolk and Egg Albumen

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Received for publication July 13, 1918

It is well known that small quantities of quinine produce in man and mammals a marked change in the metabolism of the nitrogenous constituents of the tissues. An early increase of nitrogenous bodies found in the urine is soon followed by a prolonged period of markedly diminished amounts of these substances, especially urea and uric acid. That nitrogenous food is not dissipated so rapidly but is stored in the body in some form, was shown by v. Noorden (1) in individuals kept under constant diet. In these cases the absorption of nitrogen was unchanged, its excretion was diminished and the diminution was continued for two or more days after the dosage with quinine was stopped. Our knowledge of the effect of quinine on the oxidation of the tissues includes some observations which are not easily harmonized. It has been stated that in relatively undifferentiated protoplasm—in the simpler protozoa (2) and in the ovum (3) of echinoderms—quinine retards oxidation. On these same forms, however, quinine was recognized as a general protoplasmic poison and it seems to us probable that the observed reduction of the oxidations was essentially a phase of the poisoning by the drug. In the differentiated tissues of the healthy human adult, where the methods of measuring the rate of oxidation have been much more adequate, the results have shown that there is no diminution of oxygen consumption and no decrease in the output of carbon dioxide under quinine.

Conditions present in birds offer unusual advantages for first, a study of the nitrogen conserving action of quinine in the case of a gland (the oviduct or uterus) whose sole product or secretion (egg albumen), in so far as the egg is concerned, is almost exclusively protein; second, the

similar action of quinine on the utilization of nitrogen in the last and rapid stages of growth of the ovum (yolk); third, the possibilities of testing the developmental capacities of such modified ova. Concerning the latter it may be said that the ova of pure species of doves and pigeons have been found by one of us (4) to show two kinds of eggs in respect of their metabolic and size differences and that these two levels of metabolism (which involve size as one known differential) have been correlated each with a different sex of the prospective individuals which are developed from these two kinds of eggs.

The present paper is concerned partly with the effects of the quinine on the utilization of protein by the oviduct and the ovum and partly with a demonstration of a definite change in the size of the ova developed under quinine. These ova (yolks) are smaller. The sex data derived from the incubated eggs of birds dosed precisely as were those of this series are being accumulated. Those data must, however, be considered in a later publication. Besides showing a decreased size of the ova, which decrease continues for several days after the dosage is discontinued, the data herewith given supply evidence that quinine causes a reduction from the expected or normal amounts of egg albumen (white of egg). It is highly probable that after a few weeks the amount of albumen secreted is increased considerably beyond the normal, certainly more than was present in the pre-dosing period.

MATERIAL AND METHOD

The birds used were all blond or white ring-doves (*Streptopelia risoria*, *St. alba*) or hybrids of these two forms. Only freely laying females, which with their male mates occupied separate pens, were dosed. Quinine sulfate in gelatin coated pills was used. The size of the dose was $\frac{1}{4}$ grain at first and $\frac{1}{2}$ grain later. The date of change of dosage was April 6 except when otherwise indicated in the tabulated records. These amounts were given twice daily, at about 9.00 a.m. and 6.00 p.m., except for a period of four days following the laying of the first egg of each pair of eggs. It was thought best not to handle or disturb the females at a time when this might cause the eggs to be broken or when the beginning of growth of the succeeding pair of yolks might be prevented. A pair or clutch of eggs was obtained each eight or ten days from most of the birds.

These relatively tame doves could practically always be lifted from their perches and the pellet dropped into the back of the mouth without

arousing special struggle or excitement. As a control, however, for the possible effect of handling, six of the eleven dosed birds were caught and blank-dosed twice daily from the time of the last dosage with quinine till June 16. The birds thus blank-dosed gave results wholly similar to those not thus treated. The effects observed are certainly therefore the effects of the quinine.

All eggs were examined as soon as laid—within five minutes to three hours of laying. The fresh eggs from the quininized birds were weighed and then steamed for eight minutes to coagulate the yolk and the albumen. The yolks were then immediately freed and weighed. The albumen was not separately weighed for the reason that this water-rich substance cannot be separated from both shell and yolk without an inaccuracy caused by the evaporation of water. Since the amount of shell is a relatively constant[1] and small proportion of the total weight, and showed no obvious variation in dosed[2] and not-dosed eggs, such separate weighing of the albumen is not necessary to a demonstration of the large differences observed.

Twelve yolks obtained from quininized doves were burned in a bomb calorimeter. The yolks which serve as a control for those twelve determinations were not obtained from the same birds, but from other untreated birds of similar origin and laid at a similar season of the year.

PRESENTATION OF DATA

The changes in egg size and yolk size due to quinine in all of the treated birds are indicated in tables 1 and 2. The weight averages of the six to eight eggs and yolks which immediately preceded those produced under dosage are given for each bird. Similarly the average weight of the entire group of eggs produced under dosage is next given. This group shows a pronounced decrease in egg size and yolk size in all of the treated birds. The first egg or pair of eggs produced after the quinine dosage was discontinued shows a further reduction of egg size in one-half of the group; for yolk size this further reduction occurred in

[1] The smaller the egg the greater the proportion of shell in relation to total egg size. This means that the amount of albumen in the smaller quininized eggs is even less than is indicated by the comparisons later to be made of yolk size with total egg size.

[2] Among the eggs laid by some of these birds several weeks after the dosing was discontinued a probably unusual number of eggs with weak or imperfect shells has been found. It is yet too early to be sure that this was a result of the previous treatment with quinine.

every case. Following this pair of eggs, however, the data show a progressive increase in egg size and in yolk size. At six to seven weeks after treatment was discontinued the normal egg size was exceeded in six birds and was not yet attained in four birds; for one bird the data

TABLE 1

The effect of quinine sulfate on egg size and yolk size in ring-doves

NUMBER OF BIRD	DURATION OF DOSAGE	PERIODS AS RELATED TO DOSAGE	NUMBER OF EGGS	AVERAGE WEIGHT	
				Eggs	Yolks
152	February 15 to April 30...	Before	6	8.414	1.869
		During	9	*7.711*	*1.627*
		First after	2	7.935	1.547
		Later	6	8.449	1.591
		Last	5	8.421	1.733
A347	February 19 to April 30...	Before	8	8.419	1.882
		During	10	*7.335*	*1.649*
		First after	2	6.572	1.349
		Later	4	6.934	1.593
		Last	4	7.425	1.730
903	February 23 to April 30...	Before	8	8.415	2.112
		During	8	*7.976*	*2.001*
		First after	2	8.209	1.961
		Later	8	8.388	1.899
		Last	4	8.852	1.918
24	April 5 to April 30........	Before	7	8.198	1.884
		During	0		
		First after	2	7.563	1.358
		Later	4	7.985	1.594
		Last	4	7.987	1.667
962	April 5 to April 30........	Before	8	9.073	1.982
		During	3	*8.163*	*1.754*
		First after	2	7.739	1.617
		Later	4	8.628	1.701
		Last	4	8.841	1.844

are missing, since this bird produced no eggs after the treatment was stopped. The normal yolk size at six or seven weeks had not, however, been attained in any instance. The data are available to the tenth week and the results are the same. In most cases the yolks still re-

TABLE 2

The effect of quinine sulfate on egg size and yolk size in ring-doves

NUMBER OF BIRD	DURATION OF DOSAGE	PERIODS AS RELATED TO DOSAGE	NUMBER OF EGGS	AVERAGE WEIGHT	
				Eggs	Yolks
A174	February 14 to April 30...	Before	8	8.711	2.077
		During	9	*7.354*	*1.811*
		First after	2	7.163	1.560
		Later	6	8.012	1.766
K459	February 19 to April 30...	Before	4	7.411	1.781
		During	2	*6.744*	*1.567*
		First after	2	6.270	1.253
		Later	6	7.361	1.436
		Last	4	7.786	1.534
A 22	March 23 to April 29......	Before	8	8.934	2.109
		During	2	*7.190*	*1.644*
		First after	1	7.504	1.513
		Later	6	9.078	1.958
		Last	6	9.267	1.961
E106	April 5 to April 30........	Before	6	8.417	1.901
		During	6	*8.009*	*1.765*
		First after	2	7.807	1.617
		Later	6	8.486	1.725
		Last	8	8.977	1.833
B503	April 5 to April 30........	Before	6	8.551	1.733
		During	1	*7.421*	*1.339**
E 97	April 8 to April 30........	Before	7	8.053	2.097
		During	2	*7.196*	*1.723*
		First after	2	7.459	1.422
		Later	6	7.806	1.637
		Last	4	8.093	1.743

* No later eggs to July 7.

mained much below normal size. The occurrence of excess of total egg size accoompanied by deficient yolk size in this post-treatment period is practically conclusive evidence for an excessive or supernormal production of egg albumen in this period.

The individual egg weights and yolk weights for the several abovementioned periods, as related to dosage, are given for four of the eleven birds in the left-hand columns of tables 3 and 4. It can there be seen

TABLE 3

Detailed records of egg size and yolk size under dosage with quinine. Also showing that in yolks of comparable size from the same bird the dosed yolks (columns 4, 5) are associated with smaller amounts of albumen (+ shell) than the earlier yolks (not dosed, columns 6, 7). For yolks produced long after dosage (rows designated "Later") the above rule is probably reversed.

NUMBER OF FEMALE	PERIOD WITH REFERENCE TO DOSAGE	DATE OF EGG	EGG WEIGHT	YOLK WEIGHTS		EGG WEIGHT
				Dosed	Not dosed	
152	During (February 15 to April 30)	3/7	7.592	1.621	1.615	8.095
		3/9	8.380	1.838	1.844	8.378
		3/21	7.578	1.598	1.595	7.968
		3/23	8.275	1.802	1.802	7.860
		4/1	7.792	1.578	1.590	8.040
		4/3	8.492	1.768	1.753	8.110
		4/11	7.038*	1.319*	1.330	7.530
		4/13	7.502	1.690	1.700	8.070
		4/24	6.763	1.428	1.450	7.070
	First after	5/7	7.565	1.420	1.498	7.828
		5/9	8.305	1.673	1.660	8.400
	Later	5/17	8.116	1.486	1.498	7.828
		5/19	8.545	1.585	1.595	7.968
		5/25	8.308	1.465	1.450	7.070
		5/27	9.027	1.785	1.802	7.860
		6/3	7.950	1.452	1.450	7.070
		6/5	8.748	1.773	1.753	8.110
		6/11	7.763	1.444	1.450	7.070
		6/13	8.786	1.740	1.753	8.110
903	During (February 23 to April 30)	2/27	7.820	1.980	2.005	8.035
		3/1	8.551	2.255	2.238	7.563
		3/25	7.748	1.970	1.930	8.140
		3/27	7.715	1.912	1.910	7.950
		4/5	7.810	1.945	1.923	8.215
		4/7	8.708	2.175	2.167	8.662
		4/20	7.671*	1.900*	1.890	8.430
		4/22	7.785	1.950	1.930	8.140
	First after	5/7	7.682	1.790	1.725	7.740
		5/9	8.735	2.132	2.140	8.445
	Later	5/16	7.921	1.815		
		5/18	9.300	2.255	2.238	7.563
		5/25	8.206	1.870	1.910	7.950
		5/27	9.202	2.305	2.309	9.022
		6/2	7.750	1.618		
		6/4	8.321	1.710	1.725	7.740
		6/11	7.880	1.690	1.725	7.740
		6/13	8.525	1.930	1.930	8.140

* First egg produced after increasing the dose of quinine.

† Dates apply only to the eggs whose weights are given in the adjoining column.

TABLE 4

Detailed records of egg size and yolk size under dosage with quinine. Also showing that in yolks of comparable size from the same bird the dosed yolks (columns 4, 5) are associated with smaller amounts of albumen (+ shell) than the earlier yolks (not dosed, columns 6, 7). For yolks produced long after dosage (rows designated "Later") the above rule is probably reversed.

NUMBER OF FEMALE	PERIOD WITH REFERENCE TO DOSAGE	DATE OF EGG	EGG WEIGHT	YOLK WEIGHTS		EGG WEIGHT
				Dosed	Not dosed	
A347	During (February 19 to April 30)	2/28	6.860	1.720	1.702	7.864
		3/2	7.143	1.625	1.633	7.652
		3/13	7.677	1.741	1.745	7.748
		3/15	7.863	1.795	1.775	8.240
		3/22	7.298	1.515	1.539	7.715
		3/24	7.960	1.868	1.850	8.440
		3/31	6.918	1.415		
		4/2	7.708	1.722	1.728	8.692
		4/9	7.095*	1.518*	1.539	7.715
		4/11	6.828	1.567	1.560	7.642
	First after	5/16	6.259	1.314		
		5/18	6.885	1.385		
	Later	5/26	6.999	1.475	1.480	7.480
		5/28	7.556	1.701	1.702	7.864
		6/6	6.645	1.615	1.633	8.765
		6/8	6.535	1.580	1.488	7.380
		6/25	6.965	1.565	1.560	7.642
		6/27	7.895	(1.870)	1.850	8.440
		7/4	7.357	1.600	1.560	7.642
		7/6	7.684	1.885	1.885	8.060
E106	During (April 5 to April 30)	4/7	8.835*	1.872*	1.877	8.068
		4/9	8.165	1.938	1.954	9.183
		4/16	8.038	1.708	1.771	8.800
		4/18	7.540	1.865	1.877	8.068
		4/25	7.890	1.580	1.589	8.400
		4/27	7.588	1.625	1.620	8.277
	First after	5/4	7.530	1.488	1.508	8.025
		5/6	8.083	1.746	1.778	8.172
	Later	5/12	8.415	1.623	1.620	8.277
		5/14	8.773	1.795	1.795	8.820
		5/20	8.470	1.680	1.695	8.365
		5/22	8.805	1.930	1.954	9.183
		5/28	7.895	1.550	1.584	7.900
		5/30	8.560	1.772	1.771	8.800
		6/5	8.490	1.678	1.695	8.365
		6/7	8.992	1.902	1.890	8.785
		6/13	8.995	1.815	1.842	8.885
		6/15	9.070	1.954	1.954	9.183

* First egg produced after the larger dose of quinine.

† Dates apply only to the eggs whose weights are given in the adjoining column.

that the beginning of the heavier dosage was followed by a further distinct decrease of egg size and of yolk size. It is thought unnecessary to present the corresponding data for the remaining seven birds since these data for all of the eleven birds are essentially similar.

The last two columns of tables 3 and 4 have been added in order to demonstrate that the yolks produced during dosage with quinine were not provided with the usual or normal amounts of egg albumen. The following explanation is necessary to an examination of these data. Normally, there is of course, a high correlation between the size of the yolk and the total size of the egg. Usually, though not always, the largest eggs contain the largest yolks. The reason for this rule lies in the fact that the stimulus to albumen secretion is a contact stimulus and only that portion of the gland (oviduct) is active which is in contact with the passing object. The time of passage is the same for a small as for a large object.[3] The smaller object therefore secures a smaller quantity of egg albumen. Nevertheless, two different birds do not always supply equivalent amounts to yolks of the same size; nor does the same bird, irrespective of season, age and other conditions, provide uniform quantities of albumen for yolks of equivalent size. In order to make comparisons of the amount secreted under quinine with the normal, the best that can be done is to utilize other eggs laid by the same bird.[4] In tables 3 and 4, opposite the weight of each "treated" yolk, we have therefore placed the weight of that "normal" yolk which, of all the yolks previously laid by the bird, is most nearly equal in size. The total egg weights of the two eggs which bore these two yolks can then be compared in appropriate columns of the tables.

As was stated above, the tabulated data indicate that the yolks produced under quinine did not obtain the normal quantity of egg abumen. Whether there was an actual reduction of the nitrogen present is not definitely shown by our data since it is conceivable, even though not at all probable, that the reduction occurred only or chiefly in the amount of water present in the albumen. Such an alternative assumption is made still less probable by the data, next to be mentioned, obtained by calorimetry of the yolks of the quinine series. The energy values obtained from those yolks supply evidence that the character of the yolks was left unchanged. It is hoped, however, that direct determina-

[3] These facts apply also to the shell-secreting portion of the oviduct.

[4] In tables 1 and 2 the average egg size and yolk size of the eggs laid immediately preceding the beginning of dosage were used for a similar purpose.

tions can later be made of the relative amounts of nitrogen in the normal albumen and in that produced under quinine.

Twelve yolks obtained from three of the quinine-fed doves were burned in the bomb calorimeter. When the values obtained from these eggs (table 5) are compared with those obtained from yolks produced by similar untreated females at a corresponding time of the year

TABLE 5

Bomb calorimeter determinations of the energy stored in egg-yolks from females actively dosed with quinine sulfate

NUMBER OF FEMALE	CLUTCH	DATE OF LAYING	TOTAL CALORIES	CALORIES PER GRAM MOIST WEIGHT	CALORIES PER GRAM MOIST WEIGHT IN EGGS OF COMPARABLE FEMALES NOT DOSED*	
A174	B1	3/19/18	6,540	*3,475*	3,462	3,447
	B2	3/21/18	6,225	*3,494*	3,487	3,452
	C1	3/31/18	6,156	*3,414*	3,516	3,373
	C2	4/ 2/18	6,615	*3,482*	3,485	3,422
A347	C1	3/22/18	5,100	*3,366*	3,362	3,388
	C2	3/24/18	6,321	*3,384*	3,411	3,392
	D1	3/31/18	4,710	*3,329*	3,262	3,348
	D2	4/ 2/18	5,964	*3,463*	3,344	3,392
152	C1	3/21/18	5,661	*3,543*	3,357	3,406
	C2	3/23/18	6,393	*3,548*	3,542	3,414
	D1	4/ 1/18	5,565	*3,527*	3,479	3,416
	D2	4/ 3/18	6,333	*3,580*	3,721	3,404

* Birds of similar origin and eggs taken from as nearly as possible similar dates. The two columns under this heading bear values derived from yolks of different females.

it is found that the heat values are unchanged from the normal. This is good evidence that the relation of proteins to lipoids[5] in the yolk from quininized birds was unchanged.

DISCUSSION

One situation presented by these data requires additional comment. It has been shown that after discontinuance of dosage with quinine the production of egg albumen quickly rose to and above normal, while yolk production, after ten weeks, was still below normal. The facts

[5] The energy values of yolk proteins and lipoids have been shown by one of us (Riddle, unpublished work) to bear to each other the ratio 5497 : 9020.

next to be mentioned would seem to make clear the reason for this result.

Like most tissues of the body the oviduct, flushed freely with an abundant secretion of egg albumen at the time of passage of every egg, rids itself within a few days of any trace of quinine remaining after dosage. The reverse is true for the ovum. It is practically certain that quinine is absorbed by the yolk. Quinine is not oxidized or is oxidized only to a very limited extent in the body and it is probable that the ovum has even less power than other tissues to oxidize it. The long series of smaller slow-growing ova, described in No. I of this series of papers (5), therefore absorb and continue to hold quinine within them; and this absorption and storage must be greatest for the largest of these ova and progressively less for the smaller members of the series.

Bearing in mind that the ova of the series just mentioned are libereted from the ovary in the order of their size it is clear that the most heavily dosed of these eggs were laid earliest after dosage and that the yolks with progressively less absorbed quinine form the series of yolks of progressively increasing size as found in the post-treatment period. These facts also supply the reason for the first clutch of eggs laid after dosage containing, in nine of the ten cases, a smaller yolk than was found in any egg from the dosing period; for, this pair of eggs had been dosed for a longer period than had the preceding pair and some quinine was probably still to be obtained from the blood while this pair (but not the still later pairs) was undergoing the short final period (four to five days) of rapid growth.

It thus seems probable that the presence of quinine accounts consistently for the observed fluctuations in egg albumen production and for yolk size. If—as partly noted in the introductory of this paper—earlier studies on the effects of quinine are correctly interpreted as effects on the nitrogen metabolism rather than on oxidation in general, the reduction of yolk size in the present series of ring-doves would then seem to have béen accomplished not by a raising or lowering of the general metabolism but by modification of only a part of it. Probably modifications thus limited in their scope do not provide much hope of being perpetuated in the developmental capacities of the organisms which may arise from these germs; and such modifications are apparently not of the sort which might be expected, from the results of our earlier studies, to effect a modification of sex.

The observed effects of quinine on the activity of the oviduct are fully consonant with previous observations on the nitrogen conserving

action of the drug. We are inclined to interpret the effects observed in the ovum (yolk) in essentially the same way. The presence of quinine places a check upon the usual or characteristic transformation of the nitrogenous compounds in the ova. The actual size attained by the latter is, in these cases, probably a resultant of restrictions placed upon the utilization of nitrogenous substances only.

CONCLUSIONS

When small doses of quinine sulfate are fed to laying ring-doves the yolk size and total size of the eggs produced are much decreased.

The reduction of total size is partially to be accounted for by the reduced size of the included yolks. There is, however, a clearly demonstrated additional and abnormal decrease in the amount of egg albumen produced under quinine.

These effects of quinine on yolk size are continued for at least several weeks after the dosage is discontinued. In the course of a few days or weeks, however, the eggs from treated birds contain abnormally large amounts of albumen. Ten weeks after dosage the yolks (ova) approximate but do not attain their normal size.

The relative proportion of protein to lipoid which enters into the composition of the smaller yolks produced under quinine is probably unchanged.

The known tendency of quinine to reduce the destruction of the nitrogenous components of the tissues, as measured by the recovery of nitrogen in the urine, is found to apply to the secretory activity of a gland (oviduct) whose product is entirely of protein nature.

It seems probable that the observed effects of quinine on the ova (yolks) should be similarly interpreted. The reductions and fluctuations in size of the ova are consonant with the view that, in these cases, the size attained is governed by restrictions placed upon the protein metabolism rather than upon the general metabolism.

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POST-MORTEM MELANIN PIGMENT FORMATION IN PIGMENTLESS RETINAS AND CHOROIDS OF WHITE RING-DOVES

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Received for publication July 13, 1918

INTRODUCTORY

The results described in this paper are a contribution to the following subjects: *a*, The mechanism of melanogenesis; *b*, the conditions necessary for post-mortem pigment formation; and *c*, the rôle of the hypothetical "genetic factors" in the inheritance of color.

These studies are based on the embryonic tissues of white ring-doves or of later generations of "extracted" whites from crosses with blond ring-doves. These birds have pigmented irides and non-pigmented retinas and choroids and their pigmentless choroid, skin and (approximately pigmentless) feathers separate them (*Streptopelia alba*) genetically from the next least pigmented species (*St. risoria*) of the genus. The following brief statement of the new facts obtained on the three above-mentioned subjects may be made in advance of a detailed consideration of the data:

a. A tissue which in normal living conditions can form no melanin pigment is shown to be capable of melanin formation under other conditions; the present known means of the attainment of these conditions involve the death of the tissue and a considerable post-mortem period. A certain grade or degree of tissue differentiation must have been attained before post-mortem melanin formation can occur. In the embryonic tissues studied by us the retina must have proceeded to a full 3-day stage[1] before such pigment can be made to form. It is of interest therefore that in the nearest related species—the blond ring-dove (*St. risoria*) —the pigment begins to form at this same time. This embryonic tissue

[1] The 3-day dove embryo is approximately equal in development to a 4-day chick embryo.

retains its power to form pigment post-mortem from the 3-day stage to at least the 12-day stage of development. At the hatching stage (15-day embryo) the pigment apparently does not form at moderate temperatures and without excess of oxygen. Higher temperatures with excess of oxygen have not been tried on these later stages.

b. Free oxygen is necessary for a post-mortem formation of melanin pigment. This pigment does not form in an atmosphere of CO_2. The pigment will form in a tissue killed in $HgCl_2$. It has failed to form in tissues first subjected to a temperature of 100°C.

c. Current theories of the inheritance of color, assume that these white animals—almost complete albinos—and white hybrids generally fail to produce color because discreet, segregable, germinal bases, called "color factors," fail to gain admittance into the germs which gave rise to them. Under this hypothesis the retina and choroid of these white ring-doves are and must be pigmentless because some of the necessary segregable germinal units are "absent" in the developed tissues. A modification of this hypothesis permits the assumption that the necessary factors may be present but are inactivated by the presence of other segregable germinal units designated "inhibitors." The present results demonstrate that under changed environmental conditions or under another grade or level of conditions, the tissue bears all that is necessary for pigment formation. One needs to supply no new hereditary unit nor extirpate an inhibitor to obtain an abundant supply of melanin pigment. The differences involved reveal themselves therefore in quantitative rather than in qualitative terms. The results thus offer confirmation of the view of melanin pigment formation and inheritance which was advanced by Riddle (1) in 1909.

CONSIDERATION OF PREVIOUS WORK

Bourquelot (2) and Bertrand (3) found that in dead or dying mushrooms there arises a dark color which is due to the action of an oxidase upon an aromatic substance contained in the tissue. This was the first established important fact concerning post-mortem pigment formation; at the same time it has supplied the basis for pratically all later work on the mechanism of pigment formation in living animals and plants. Nearly all of the later work has dealt with pigment formation in the living organism or (chemical studies) *in vitro*. So far as known to us, only the few studies made in Meirowsky's laboratory have considered the formation of melanin pigment in animal tissues after

death. Those studies, however, furnish important information on the subject. The first of these publications appeared in 1908. For the purposes of the present paper only the following brief statement concerning the results obtained in this earlier work need be made.

Meirowsky (4) first demonstrated that melanin pigment is formed after death *a*, in human skin from various portions of the body and in scar tissue; *b*, in the embryonic epidermis of the cat, pig and dog.[2] Concerning the mechanism of post-mortem pigment formation Meirowsky showed *a*, that the first appearance of such pigment requires a considerable period of time;[3] *b*, at 56°C. the pigment was produced in human skin in about one-half the time required for its production when kept at 36°C.; *c*, the pigment was formed in pieces of skin that had been subjected to "boiling" in water. Meirowsky favored the view that the melanin production is brought about by enzyme action.

The above studies were continued by Königstein (5) who more carefully studied the conditions under which post-mortem pigment formation occurs. It was found *a*, that pieces of skin immersed in physiological salt solution do not "blacken" at a temperature of 37°C.; *b*, pieces of boiled skin and skin fixed in 1 per cent sublimate or 10 per cent formalin, when dried in Petri dishes and placed for "several hours" at 56°C., become "coal black;" *c*, the "drier the preparation and the higher the temperature, the greater is the degree of pigmentation." Königstein speaks against a pure "ferment" theory being able to account for the results and cites the probable destruction of the enzymes by boiling.

Two further results of Königstein's study will be specially noted and a word of discussion added. *d*, It was found that pieces of skin boiled in alcohol or ether would form the pigment but pieces boiled in acetone would not (except when later heated in soda solution). Since alcohol and ether dissolve lipoids and acetone precipitates them, Königstein inclines to the conclusion that the lipoids stand in a close relation to the formation of the pigment. We may remark that this

[2] The title of Meirowsky's paper indicates observations on pigment formation in the *eye* as well as the skin, but *post-mortem* pigment formation seems not to have been observed in the eye. His study was, in fact concerned less with the mechanism of pigment formation, post-mortem or otherwise, than with other questions; among these, the origin of pigment independently of melanoblasts (chromatophores), the migration of melanoblasts, the source and mother substance of melanin, etc.

[3] Less time was required in two cases: Skin from a patient dead of Addison's disease and pieces of skin which before death were subjected to Röentgen rays.

is not a necessary inference from the data and that the facts now known concerning the chromogens necessary for melanin formation attest the much greater importance of certain constituents of protein. *e*, Finally, the rôle of oxidation and reduction was partially investigated with essentially negative results. In view of the results of the present writers the following statement is of interest:

Um zu ermitteln ob ein oxydation prozess die Pigmentbildung begünstigt wurden Hautstüche durch 48 Stunden hindurch in geschlossenen Kolben in je einer CO_2O, or H, Atmosphere ausgesetz ohne dass jedoch in einem der 3 Versuche eine Veränderung zu bemerken war.

Our data demonstrate that, at least for the tissues studied by us, forty-eight hours is not time enough to obtain the pigmentation; all of three days is required. We have demonstrated also that the pigment studied by us can not form at all in CO_2, while in all percentages of oxygen, between the amount present in air and 100 per cent, the pigment forms if a period of three days or slightly longer is given for the test.

In a still further continuation of the above studies Winternitz (6) made the interesting observation that drying the skin produces a dark coloration in it which is removed when the dried colored skin is later soaked in water. The meaning of this observation is not clear to us. This investigator also treated pieces of skin with various oxidizing and reducing agents and concluded that

reagentien von nicht bedeutender Konzentration hindert das Eintreten des postmortalen Verfärbung nicht.

Further, from the skin of rabbits a substance was obtained which was thought to be chemically similar to adrenalin. This last-named observation is of some interest in connection with a post-mortem formation of a blackish pigment in the adrenal itself as was noted by Meirowsky (7). The latter author concluded, however, that the pigment produced in these adrenals is not melanin because, unlike that formed in the skin, it is not in "granular" form. This objection seems unimportant. In defining "melanins," first consideration may well be given to the chemical nature of the pigment and its mother substance (chromogen) and to the nature of the processes concerned in the transformation of the latter into a colored compound.[4]

[4] Abderhalden and Guggenheim (1908) have shown that adrenalin, under the influence of *tyrosinase*, produces dark or dark red flocculi.

PRESENTATION OF DATA

The present studies were undertaken primarily because one of us (Riddle) had occasionally observed in connection with other studies, during two or three previous years, the presence of retinal pigment in some dead embryos of birds (*St. alba*, and some "extracted" whites) known to be incapable of forming such pigment during life. In several of these cases it had been noted that the pigment was found only in a single eye and that this eye was usually or always the one (right) in contact with the air chamber of the egg.[5] It had also been observed that such unexpected pigment was found only in embryos that had died some days before the egg was opened for examination; and further, such eggs seemed often to have especially large air cavities, i.e., these eggs had lost an unusual amount of water and had admitted a correspondingly unusual amount of air. For the purposes of extensive studies being conducted by the senior author, it became necessary to

TABLE 1

Pigment formation from eggs desiccated (over H_2SO_4*) while kept for a period in an incubator (103° F.); later incubation under birds*

PARENTS	EGGS LAID*	DATE TO DESICCATOR; PREVIOUS RATE OF LOSS OF WEIGHT IN MILLIGRAMS PER HOUR	SERIES OF DAILY WEIGHINGS; AND RATE OF LOSS IN MILLIGRAMS PER HOUR					REMOVED FROM DESICCATOR	DATE OF HATCHING OR OF EXAMINATION	PIGMENT IN	
			I	II	III	IV	V			Iris	Retina-choroid
91	4/24	4/26; 5.4	4.9	4.7	5.8	5.2	5.0	5/6, p.m.	Alive 5/14	Traces	None
188a	4/27	4/28	4.4	3.3	3.6	4.0	3.8	5/6, p.m.	Hatched 5/13	Inner ring	None
	4/29	4/29	4.5	4.6	4.8	4.9	5.2	5/9, p.m.	Hatched 5/15	Inner ring	None
2	4/27	4/28; 3.6	4.5	4.0	4.1	3.8	3.7	5/6, p.m.	Partly hatched 5/14	Inner ring	None

* Five eggs were used; one (4/26 of 91) proved infertile.

[5] Embryos that have developed more than five or six days lie in such position as to imbed the left eye against the egg yolk and the right eye against the inner shell-membrane limiting the air chamber. This is the position of the right eye in all later embryonic stages in those eggs which have lost very considerable quantities of water. It seemed conceivable that the actual tissues of the right eye might thus be robbed of moisture during or preceding the formation of the pigment.

learn something of the unexpected and unexplained presence of retinal or choroid pigment in dead embryos derived from parents whose offspring should be incapable of producing it. The observations which have been indicated above supplied the reasons for conducting the present observations and experiments along the lines reported here. The known facts of melanogenesis were further reason for an examination of the relation of oxygen (and of carbon dioxide) to the process.

A group of eggs was placed for a period of eight to ten days in an incubator and desiccated at approximately twice the normal rate.[6] These eggs were later placed under birds and hatched (table 1). The eyes of these birds had the normal distribution of pigment, this being present in the iris and wholly absent in the retina and choroid. A considerable number of embryos, of late and full-term stages, were killed previous to the beginning of these experiments in order to supply control data. It seems wholly unnecessary to publish these data since those of table 1 and of all individuals of other tables which survived in the experiments are quite identical with the control.[7] The amount of desiccation obtained in this experiment had therefore no effect upon the pigmentation of embryos which continued to live.

The desiccation of a second group of eggs was carried out, but in connection with incubation in a 100 per cent oxygen atmosphere (table 2). It will be seen that practically all of the embryos died very soon after being placed in the oxygen. A comparison of the ages of the embryos when placed in oxygen, and the estimated ages of the embryos when these were examined several days later, shows conclusively (see columns 5 and 6) that death was caused by the gas[8] into which they were placed. The important fact to be observed in the data of this table is that the three embryos which had attained an age of three to four days or more were the only ones which formed pigment in the eye, and that these three formed pigment not only in the iris where it occurs normally in life, but also in the retina-choroid where it does not form during life. A 2-day embryo failed to form any pigment in either iris or retina and a 3- to 4-day embryo had formed only traces in the normally pigmentless retina. Further, at the time of examination these embryos had been dead for eight days, during three to five of which they had been in 100 per cent oxygen.

[6] This rate of loss under normal incubation is considerably higher during the first twelve to twenty-four hours after laying than on subsequent days.

[7] The control data for post-embryonic stages are given in table 10.

[8] The oxygen gas doubtless contained some impurities. Oxygen prepared by the Linde Air Products Company was used in all our experiments.

TABLE 2

Pigment formation from eggs desiccated (over H_2SO_4 in incubator) for short periods, then continued desiccation and incubation in (nearly) 100 per cent oxygen (fatal dosage) for a few days; still later incubation of some of the dead embryos (first 4 eggs of table) under birds

Parents	Eggs laid	Date to desiccator	Rate of loss during 5 days	Age when O_2 was added	Age of embryo	Date of record	Time after death	Time in O_2	Pigment in: Iris	Pigment in: Retina-choroid
				days	*days*		*days*	*days*		
91	5/3	5/3 ; 5:40 p.m.	5.2	7.0	7	5/18	8.0	3.0	Very black	Very black
	5/5	5/5 ; 1:30 p.m.	6.5	5.0	4–4.5	5/18	8.0	3.0	Black	Black
261	5/6	5/7 ; 12:30 p.m.	3.2	4.0	3–4	5/18	8.0	5.0	Black	Traces
	5/8	5/8 ; 10:30 a.m.	7.7	2.5	2	5/18	8.0	4.0	*	*
161d	5/8	5/9 ; 1:30 p.m.	3.2	2.0	0	5/15	Infert.			
	5/10	5/10; 2:30 p.m.		0.5	Broken, p.m.	5/11	Infert.			
188a	5/8	5/8 ; 6:00 p.m.	4.5	2.0	2.5	5/17	7.0	7.0		
	5/10	5/10; 10:15 a.m.	6.3	0.5	1 (?)	5/18	7.0	7.5		
170	5/9	5/9 ; 11:30 a.m.	5.8	1.0	1–2	5/15	5.0	5.0		
	5/11	5/11; 10:30 a.m.	7.8	0.0	1 (?)	5/18	6.0	7.0		
91	5/12	5/12; 6:00 p.m.	6.2	0.0	1 (?)	5/18	5.0	6.0		
	5/14	5/15; 11:30 a.m.	9.8†	1.0	1–1.5	5/18	2.5	3.0		
261	5/15	5/15; 6:00 p.m.	7.2†	1.5	1.5‡	5/19		3.8		
	5/17	5/18; 12:00 m.	6.0†	0.0	1.5–2	5/19	Alive§	2.0		

* The blank spaces indicate no pigment, but in addition they direct attention to the fact that the cause of this is connected with the very immature and undifferentiated state of the embryo. Neither normal nor post-mortem pigment formation has been observed in embryos of less than 3 days of development.

† The period here was for less than 5 days; the rate of loss is usually greater during the first days after laying. The larger figures of this column are usually those for *larger* eggs; the second egg of the pair is larger in most cases.

‡ The embryo (if ever present) had wholly disappeared; this figure is for the stage attained by the *vascular area.*

§ Very little blood in vascular area.

A similar result was obtained on another group of embryos from eggs which were not desiccated (table 3), but otherwise received the same treatment as the above. Desiccation therefore was not a real factor

TABLE 3

Pigment formation from eggs incubated but not desiccated for a short period before being placed temporarily in a (nearly) 100 per cent oxygen atmosphere (no method used for the absorption of CO_2, and no desiccation); later incubation under birds

Parents	Eggs laid	Date to 100 per cent O_2	Number hours in 100 per cent O_2	Age when O_2 was added	Age of embryo	Date examined	Time for post-mortem pigment formation	Pigment in: Iris	Pigment in: Retina-choroid
				days	days		days		
116m	5/17	5/25; 9:30 p.m.	21.5	8.0	8–9	5/30	4	R., very black; L., traces	Very black; Traces
	5/19	5/25; 9:30 p.m.	21.5	6.5	6.5	5/30	4	R., (?); L., traces	(?); Traces
170	5/19	5/24; 5:00 p.m.	50.0	5.0	5.0	5/29	4	R., traces; L., more	None
	5/21	5/24; 5:00 p.m.	50.0	3.5	4–4.5	5/29	3–4	None	None
188a	5/19	5/24; 5:00 p.m.	50.0	5.0	5.5–6	5/30	5	Black	Black
	5/21	5/24; 5:00 p.m.	50.0	3.5	5–5.5	5/29	2–3	None	None
91	5/21	5/25; 5:30 p.m.	26.0	4.0	4.0	5/30	4	Black	Black
	5/23	5/25; 5:30 p.m.	26.0	2.5	2.5	5/30	4		
117a	5/23	5/25; 5:30 p.m.	26.0	2.0	3.5	5/26	Alive*	(?)	None

* This embryo was probably alive when egg was opened May 26 at 7:30 p.m. This error resulted because of the very *few and indefinite blood vessels* present; when candled it seemed infertile. This case and a number of similar instances met with later possibly indicate that the regulatory mechanisms of the early developing embryo include the power to restrict the number of extra-embryonic blood vessels (and the quantity of blood?) when exposed to a highly oxygenated atmosphere. On the other hand, these changes may be due to "poisonous" effects of the gases used.

in the cases considered above. It doubtless accompanies a ready ingress of air or of oxygen. Table 3 shows that retinal pigment formed in four of the six embryos which had attained an age of four days or more before death and which in addition had been dead for three or

TABLE 4

Pigment formation in embryos temporarily subjected to abnormally high (oven, 50° to 57°C.) and low (ice box, about 6°C.) temperatures. It was erroneously supposed that the temperature used would kill all embryos within the time limits

PARENTS	EGGS LAID	NUMBER OF HOURS EXPOSED	KILLED OR SURVIVED	AGE WHEN EXPOSED	AGE ATTAINED BY EMBRYO	DATE EXAMINED	TIME POST-MORTEM	PIGMENT IN: Iris	PIGMENT IN: Retina-choroid
(Ice box, about 6°C.)									
				days	*days*		*days*		
261	5/24	19.0	Killed	11.0	11.0	6/15	11	R., traces*; L., none	Traces*; None
	5/26	19.0	Killed	9.0	9.0	6/15	11	R., dark*; L., traces	Dark*; (?)
188a	5/28	19.0	Killed	6.5	6.0	6/15	12	None†	None†
	5/30	19.0	Survived	5.0	13.0 (? alive)	6/15	0	Trace†	None†
2	5/28	19.0	Killed	6.5	7.0	6/15	5	R., inner ring; L., traces	None
	5/30		Survived (?)	5.0	13.0	6/15	3	Inner ring	None
161	5/29	19.0	Survived	5.5	17.0 (full-term + 2 days)		0	Inner ring	None
	5/31	19.0	Survived	4.0	hatched	.	0	Dark on 7/18	None
161d	5/30	19.0	Killed	5.0	4.5	6/15	11	R., dark; L., none	(?); None
Oven, 50° to 57°C.									
188a	6/5	19.5	Killed	10.5	10.0	6/21	6	Dark	Black
	6/7	19.5	Killed	9.0	9.0	6/21	5	R., dark; L., none	Traces; None
116m	6/5	19.5	Killed	10.5	10.0	6/21	6	R., inner ring; L., traces	Dark; None
	6/7	19.5	Killed	9.0	9.0	6/21	5	R., dark; L., none	Dark; None
117a	6/6	19.5	Killed	9.5	9.0	6/21	6	Dark	Dark
	6/8	19.5	Killed	8.0	8.0	6/21	5	R., dark; L., traces	Dark; None

* From 6/10 to 6/15 this egg was kept in a refrigerator; probably little or no pigment could form while kept at this low temperature.

† From 6/12 to 6/15 this egg was kept in a refrigerator.

TABLE 4—*Continued*

PARENTS	EGGS LAID	NUMBER OF HOURS EXPOSED	KILLED OR SURVIVED	AGE WHEN EXPOSED	AGE ATTAINED BY EMBRYO	DATE EXAMINED	TIME POST-MORTEM	PIGMENT IN	
								Iris	Retina-choroid
Oven, 50° to 57°C.—*Continued*									
				days	*days*		*days*		
91	6/9	19.5	Killed	6.5	6.5	6/21	5	R., black	Black
								L., black	Traces
	6/11	19.5	Killed	5.0	5.0	6/21	5	R., dark	Dark
								L., traces	Traces
2	6/9	19.5	Survived	6.5	11.5	6/21	0	Inner ring	None
	6/11	19.5	Survived	5.0	9.5	6/21	0	Inner ring	None

more days. The two exceptions (young of 170) were embryos of five days or less. An embryo of two and a half days, dead for four days, and a 3.5-day live embryo had formed no retinal pigment.

Two groups of embryos were next killed, and otherwise not treated, in order to learn whether the pigment would form without excess (not more than in air) of oxygen and without desiccation (table 4). One group was killed by low temperature (6°), the other by high temperature (50° to 57°). It will be noted that a few embryos survived these rather extreme temperatures in which they were kept for nineteen hours. Of the thirteen birds of these two groups which attained an age of more than three days, and also remained dead more than three days before examination, ten formed retinal pigment; one was questionable and two failed to form such pigment. The embryos that failed were of six and a half days. None of the five embryos which remained alive showed any trace of retinal pigment.

A group of embryos was next placed in a 50 per cent oxygen atmosphere. The data of table 5 indicates that nine of the ten embryos treated were killed by this degree of concentration of the gas. That all of the embryos had continued to live in the gas for a period of one to two days is shown, however, by an examination of columns 6 and 7 of the table. The three embryos of more than eight days of development all produced retinal pigment; two of six to six and one-half days and one of three to three and one-half days failed to do so. All had four to five days post-mortem as the period during which the pigment might

form. Three embryos of three days of development or less formed no pigment.

Another group of quite young embryos, of one and one-half to three days, were placed during four to eight days in 35 per cent oxygen;

TABLE 5

Pigment formation in embryos from eggs normally incubated for a period and then permanently transferred to (nearly) 50 per cent oxygen in incubator (no desiccation)

PARENTS	EGGS LAID	DATE (12:00 M.) PLACED IN O_2	TIME OF EXPOSURE TO O_2	KILLED OR SURVIVED	AGE WHEN PUT IN O_2	AGE REACHED BY EMBRYO; ALL EGGS EXAMINED JUNE 29, P. M.*	NUMBER OF DAYS POST-MORTEM	PIGMENT IN: Iris	PIGMENT IN: Retina-choroid
			days						
188a	6/13	6/23	6	Killed (?)	10.0	12	4.0	R., none (?)	Black
								L., none	None
	6/15	6/23	6	Killed (?)	8.0	10	4.0	R., none	Traces
								L., none	None
261	6/16	6/23	6	Killed (?)	7.0	8	5.0	R., black	Black
								L., traces	Traces
	6/18	6/23	6	Killed (?)	5.0	6–6.5	4.5	R., trace	None
								L., none	None
91	6/18	6/23	6	Killed (?)	5.0	6–6.5	4.5	R., traces; L., none	None
	6/20	6/23	6	Killed (?)	3.0	3–3.5	5.5	None	None
170	6/21	6/23	6	Killed (?)	2.0	3	5.0		
	6/23	6/23	6	Killed	0.1	1	5.0		
188a	6/22	6/23	6	Killed	1.0	2–2.5	4.5		
	6/24	6/26†	3	Survived	2.5	5–5.5	0.0	None	None

* On the last day of incubation, June 28–29, the temperature of the incubator got too high—about 109°F.; later examination of the embryos of this series showed, however, that *all* had died before this period.

† This egg in O_2 at 5:00 p.m.

this percentage, however, at one time became less than normal and killed six of the eight embryos. The embryos ranged from three to ten days of development; the time post-mortem for pigment formation was only one day (two days in one case) and no retinal pigment was

formed in any case (table 6). A very striking fact concerning this series is that there was also *no pigment in the irides of any of this group when they were examined.* This pigment had certainly been present earlier in the irides of the (seven) embryos which were of more than four days of development. Since all suffered a *lack* of oxygen at the

TABLE 6

Eggs normally incubated for a short period and then permanently transferred to (nearly) 35 per cent oxygen in incubator. On July 6 and 7 there was a deficiency of oxygen,—less than in the air—which caused the death of 6 of the 8 embryos*

Parents	Eggs laid	Date placed in O_2	Time of exposure to O_2	Killed or survived	Age when put in O_2	Age reached by embryo	Date of examination	Number of days post-mortem	Pigment in	
									Iris	Retina-choroid
			days							
161c	6/26	6/29, p.m.	8	Killed (?)*	3.0	9–10	7/7	1	None	None
	6/28	6/29, p.m.	8	Killed (?)	1.5	8.0	7/7	1	None	None
116m	6/27	6/29, p.m.	8	Killed (?)	2.0	8–9	7/7	1	None	None
	6/29	6/29, p.m.	8	Killed (?)	0.4	6.5–7	7/7	1	None	None
91	6/27	6/29, p.m.	8	Survived	2.0	9	7/7	0	None	None
2	6/30	7/3 , p.m.	4	Killed	3.0	4–5	7/7	2	None	None
188a	6/30	7/3 , p.m.	4′	Killed (?)	3.0	5–6	7/7	1	None	None
	7/2	7/3 , p.m.	4	Killed (?)	1.3	3–4	7/7	1	None	None

* This was the *initial* percentage of O_2; it was of course continually diminished through the respiration of the several embryos. At the end of the first three days the oxygen chamber supported a flame better than the air and was then refilled. On July 7, however, at the end of a four-day period (and with larger embryos) there was a decided *deficiency* of O_2 in the chamber. On July 3 concentrated NaOH solution was placed in the bottom of the chamber to absorb the output of CO_2; hitherto the CO_2 had been permitted to accumulate. When the chamber was opened on July 7 it showed a partial vacuum, due to the absorption of CO_2.

time of death and for some time thereafter it is reasonable to ask whether this lack of oxygen caused an actual disappearance of pigment already formed. That this was almost certainly the case is shown by the data of table 9.

Table 7 supplies details for another group of embryos kept in 35 per cent oxygen. Four of these birds hatched in the oxygen chamber;

TABLE 7

Eggs normally incubated for a period and then permanently transferred to (nearly) 35 per cent oxygen in an incubator—the O_2 supply being automatically maintained at 35 per cent

PARENTS	EGGS LAID	DATE PLACED IN O_2	TIME OF EXPOSURE TO O_2	KILLED OR SURVIVED	AGE WHEN PUT IN O_2	AGE REACHED BY EMBRYO	DATE OF EXAMINATION	NUMBER OF DAYS POST-MORTEM	PIGMENT IN Iris	PIGMENT IN Retina-choroid
			days							
161	7/2	7/11, p.m.*	7	Hatched	9.0	Hatched	7/18	0	Inner ring	None
	7/4	7/11, p.m.	7	Hatched	7.0	Hatched	7/18	0	Inner ring	None
91	7/6	7/11, p.m.	11	Hatched	5.0	Hatched	7/22	0	Inner ring	None
116m	7/7	7/11, p.m.	8	Killed (?)	4.0	10.5–11	7/19	1.0	None	None
161d	7/8	7/11, p.m.	12	Died, Hatched	3	Hatched	7/23	0	Diffuse	None
125y	7/9	7/11, p.m.	8	Killed	2	7–8	7/19	2	None	None
188a	7/9	7/11, p.m.	12	Killed	2	8–9	7/23	3	R., traces; L., none	Dark traces
	7/11	7/11, p.m.	8	Dead (?)	0	8–9	7/19	1	None	None
117a	7/10	7/11, p.m.*	12	Killed	1	7	7/23	6	Traces (?)†	Dark
	7/12	7/12, p.m.	7	Killed	0.3	5	7/19	2	None	None
161c	7/10	7/12, p.m.	3	Killed	2	3	7/15	2	None	None
	7/12	7/12, p.m.	11	Killed	0.3	6–6.5	7/23	4.5	(?)	(?)
116m	7/13	7/19, p.m.	6	Killed‡	6	9	7/25	3	R., dark; L., none	Dark
	7/15	7/19, p.m.	6	Killed	4	7	7/25	3	(?)	Traces
262	7/15	7/19, p.m.	6	Killed	4	5–6	7/25	4	Traces	(?)†
125y	7/17	7/19, p.m.	6	Killed	2	4–4.5	7/25	3.5	None	None
	7/19	7/19, p.m.	6	Killed	0.3	3.5	7/25	2.5	None	None
91	7/18	7/19, p.m.	6	Killed	1	4–4.5	7/25	1.5	None	None

* The eggs of this table which were in the oxygen chamber, 7/11 m. to 7/12 p.m. were in approximately 40 per cent oxygen.

† This embryo was considerably macerated and its tissues fell apart before the examination could be completed.

‡ On 7/24 the automatic oxygen supply did not work properly and there was no excess of O_2 in the chamber. The six embryos then in the chamber were all dead at that time as is shown by the figures for the ages attained by them.

they had pigment in the iris and none in the retina. Eight older embryos[9] had three or more days for post-mortem pigment formation; five of them formed retinal pigment, one failed and two were not successfully examined. Seven had less than three days post-mortem for pigment formation and none formed the pigment; but these, like the case noted immediately above, also *failed to show pigmented irides* and this raises again the question whether a temporary *deficiency* of oxygen may not have caused the disappearance of pigment already formed. For four of these embryos[9] (last two rows or table) we had reason to suspect a deficiency of oxygen soon after their death. It is possible that an unobserved deficiency occurred also earlier; if so this may account for the five similar cases to be seen in the entire table. All of the nineteen embryos of this series attained the age of three or more days before death.

A further series of ten embryos was very carefully, and we believe successfully, kept during ten to twelve days in a 40 per cent oxygen atmosphere. One bird hatched and another partly hatched in the oxygen chamber. All lived very much longer than when placed in higher percentages of oxygen and possibly all would have survived except for a single but considerable aberration of temperature in the incubator (table 8). All except one (two and a half days) of these birds attained an age of eight to fifteen days. Autopsies were made near to what we now recognize as the minimum time, post-mortem, which is necessary for pigment formation. This period for one embryo was four days; it formed a heavy black retinal pigment. For three embryos the period was three days; one formed the pigment and two failed. For three other embryos the period was two days and none formed the pigment. For one bird the period was one day and no pigment was formed. Finally, for two this post-mortem period was less than one day and no pigment was formed.

Tables 3 to 8 show that it was possible to differentiate in the two eyes the amounts of retinal pigment formed post-mortem in fourteen cases. In every case the right eye (which, as earlier noted, is the eye in close contact with the oxygen of the air chamber of the egg) had more pigment. The amount of pigment borne in the irides was differentiated in eighteen embryos; in seventeen of these the right iris had more pigment and in one case it had less. In the exceptional case (table 3) it

[9] The first egg of table 8 is included in this statement.

TABLE 8

Eggs normally incubated for a period and then permanently transferred to (nearly) 40 per cent oxygen in incubator—the O_2 supply being automatically maintained at 40 per cent

PARENTS	EGGS LAID	DATE PLACED IN O_2	TIME OF EXPOSURE TO O_2	KILLED OR SURVIVED	AGE WHEN PUT IN O_2	AGE REACHED BY EMBRYO	DATE EXAMINED	NUMBER OF DAYS POST-MORTEM	PIGMENT IN: Iris	PIGMENT IN: Retina-choroid
			days							
161	7/18	7/18, p.m.	6	Killed*	0	3.0	7/24	3	None*	None
	7/20	7/25, p.m.	10	Killed (?)†	5	13.0	8/4	2	Inner ring	None
117a	7/19	7/25, p.m.	10	Killed (?)	6	13.0	8/4	3	Diffuse	None
	7/21	7/25, p.m.	10	Killed (?)	4	13–14	8/4	2	Diffuse	None
91	7/20	7/25, p.m.	10	Hatched	5	Hatched	8/4	0	Inner ring+	None
188a	7/20	7/25, p.m.	10	Killed (?)‡	5	15.0	8/4	0	Inner ring	None
	7/22	7/25, p.m.	3	Killed (?)	3	2.5	7/28	3	None	None
161d	7/20	7/25, p.m.	10	Killed (?)	5	12–13	8/4	2	Diffuse	None
	7/22	7/25, p.m.	10	Killed (?)	3	11–12	8/4	1	Diffuse	None
116m	7/25	7/28, p.m.	12	Killed	3	9.0	8/9	3	R., very black; L., dark	Very black; None
	7/27	7/28, p.m.	12	Killed	1	7–8	8/9	4	R., very black; L., dark	Very black; None

* This egg was treated with others recorded in table 7; 35 per cent of O_2 was used for that series.

† On the night of 7/28–29 the temperature of the oven reached 109°F. Although none of the embryos died at that time it is possible that the birds here marked "killed (?)," were at least weakened by this high temperature.

‡ This bird pricked the shell but died before complete hatching.

was not recorded whether the position of the embryo in the egg was normal.

Another fragment of data bearing on this topic is supplied by table 9. In this case twenty-four embryos were killed by a deficiency of oxygen (or poisoning with 100 per cent CO_2). In two cases the amounts of iridial pigment in the two eyes could be distinguished. Here one

right iris showed more pigment and one showed less. It will be noted in that group that only four of twenty-four pairs of irides showed any pigment although it is practically certain that all of these irides contained pigment at the time of death. When killed by CO_2 and kept in CO_2 for periods of five to eight days not more than six of the forty-eight irides showed any trace of pigment.

The data of table 9 were obtained with the purpose of learning whether post-mortem pigment formation can occur in the absence of oxygen. The data previously obtained had shown that such pigment can form in oxygen percentages ranging between that present in normal air and 100 per cent. Three separate groups of embryos—as indicated by the divisions on the table—were separately used in this study. Having previously learned that the stage reached by the embryo must be three or more days, only embryos from four to nine days of development were used. The table makes clear the fact that in the absence of oxygen not a single case of pigment (choroid-retinal) formation occurred.

Observations on embryos at the hatching stage (fourteen to fifteen days) are not complete. Three groups of these embryos kept in air at 30°, 50° and 58° respectively, failed to show a formation of pigment. Higher percentages of oxygen and higher temperatures have not been tried. These experiments were not precisely parallel to those made with younger embryos since the eggs were partly opened to make sure that the bird was alive; in some cases the mature embryos were removed entirely from the shell. All these embryos dried faster than the earlier embryos although in the lot kept at 58° moisture was continually kept in the bottom of the containing chamber. It is perhaps conceivable that the drier cornea and sclera greatly lessened the oxygen supply to the choroid and retina. It is also conceivable that the later and more complete differentiation of the outer layers of the eye greatly restrict the diffusion of oxygen into the eye.

In view of Meirowsky's record of the formation of the pigment in boiled pieces of skin, the following incomplete results are perhaps of interest. Six eggs containing embryos were subjected for fifteen minutes to a temperature of 100° to 107°. These eggs were then kept over moisture at a temperature of 52° to 55°C. during four and six days. When these embryos were examined they were found surrounded by considerable moisture and no pigment had formed post-mortem in any case. Since the heating of the eggs and the presence of moisture in considerable amounts may have lessened the amount of available

TABLE 9

Eggs normally incubated for a period and then permanently transferred to (nearly) 100 per cent CO_2 in incubator. Three separate sets of eggs used, as indicated by the divisions of the table

Parents	Eggs laid	Date placed in O_2	Time of exposure to O_2	Killed or survived	Age when put in O_2	Age reached by embryo	Date examined	Number of days post-mortem	Pigment in: Iris	Pigment in: Retina-choroid
261	7/31	8/9, p.m.	6	Killed	9	9	8/15	6	None	None
	8/2	8/9, p.m.	8	Killed	7	7	8/17	8	R., inner ring– L., none	None
91	8/1	8/9, p.m.	6	Killed	8	7	8/15	6	None	None
	8/3	8/9, p.m.	8	Killed	6	5–6	8/17	8	None	None
188a	8/1	8/9, p.m.	6	Killed	8	7.5–8	8/15	6	None	None
	8/3	8/9, p.m.	8	Killed	6	6	8/17	8	None	None
161	8/1	8/9, p.m.	6	Killed	8	7–7.5	8/15	6	Trace ? (inner)	None
	8/3	8/9, p.m.	8	Killed	6	6	8/17	8	Trace ? (inner)	None
116m	8/2	8/9, p.m.	6	Killed	7	6.5–7	8/15	6	None	None
	8/4	8/9, p.m.	8	Killed	5	5	8/17	8	None	None
161d	8/4	8/13, p.m.	8	Killed	9	8	8/21	8	None	None
	8/6	8/13, p.m.	8	Killed	7	7	8/21	8	None	None
2	8/4	8/13, p.m.	8	Killed	9	9	8/21	8	R., none L., trace? (inner)	None
	8/6	8/13, p.m.	8	Killed	7	7	8/21	8	None	None
170	8/7	8/13, p.m.	8	Killed	6	5–5.5	8/21	8	None	None
	8/9	8/13, p.m.	8	Killed	4	4	8/21	8	None	None
116m	8/12	8/21, p.m.	5	Killed	9	8–8.5	8/26	5	None	None
	8/14	8/21. p.m.	5	Killed	7	7	8/26	5	None	None
261	8/13	8/21, p.m.	5	Killed	8	7–7.5	8/26	5	None	None
	8/15	8/21, p.m.	5	Killed	6	5.5–6	8/26	5	None	None
161	8/14	8/21, p.m.	5	Killed	7	6	8/26	5	None	None
	8/16	8/21, p.m.	5	Killed	5	4.5–5	8/26	5	None	None
188a	8/15	8/21, p.m.	5	Killed	6	5.5	8/26	5	None	None
	8/17	8/21, p.m.	5	Killed	4	4	8/26	5	None	None

oxygen, this test is unsatisfactory. The experiment was therefore repeated and modified by removing a part of the shell from the air space in order to permit an ingress of air. These embryos, however, became very dry within forty-eight hours. No pigment had formed; but in view of our results on the younger embryos the time was not sufficient.

TABLE 10

Pigment in eyes of control birds killed at various ages

PARENTS (PAIRS)	YOUNG	AGE	PIGMENT IN		PARENTS (PAIRS)	YOUNG	AGE	PIGMENT IN	
			Iris	Retina-choroid				Iris	Retina-choroid
		days					*days*		
170	1	145	Dark	None	91	9	59	Dark	None
	2	100	Dark	None		10	49	Dark	None
	3	98	Dark	None	161	11	44	Dark	None
	4	74	Dark	None		12	42	Dark	None
188a	5	206	Dark	None	261	13	49	Dark	None
	6	91	Dark	None	262*	14	52	Dark	None
	7	80	Dark	None	116m	15	40	Dark	None
	8	70	Dark	None	118a	16	68	Dark	None

* Parents similar to birds of pair 261. All of the young produced by this pair were of white color. One egg from this pair was used in the studies outlined in table 7.

In a last examination of this point three pairs of 6- to 8-day embryos were used. The three 8-day embryos were killed at 103°; the 3- to 6-day embryos were killed by immersion in a saturated solution of $HgCl_2$, care being taken to prevent the air space of the egg from coming in contact with the solution; the uncoagulated membrane would therefore continue to permit the ingress of oxygen into these eggs. The six embryos were all examined at the end of four days. The three killed at 103° showed no retinal pigment; the three embryos killed in $HgCl_2$ all showed heavy formations of retinal pigment. In our studies there has been no trace of pigment formation in any embryo subjected to a temperature of 100° or more for periods of five to fifteen minutes.

Our observations and data indicate that the post-mortem pigment formation begins at the inner (pupillary) edge of the iris, later appears in the outer iris and still later in the entire retina-choroid area. This series has an interesting parallel in the order in which the pigment arises in the normal ontogeny. All of the several earlier stages (less than 3-

day embryo) of these living white embryos have no pigment in the iris. When the pigment first appears it forms an inner ring to the iris which is later extended to the outer iris. In these doves the retina-choroid area remains pigmentless throughout life but in their closest relative, the blond ring-dove (*St. risoria*), the formation of iridial pigment is *followed* by the pigmentation of the retina-choroid area A histological study of the macerating[10] ocular tissues bearing the pigment was attempted in only unfixed and smear preparations. Aside from the precise placement of the pigment its solubilities and appearance in fresh preparations under the microscope show that it is unquestionably melanin ocular pigment.

POST-MORTEM PIGMENT FORMATION AND THE POSTULATION OF THE ABSENCE OF GENETIC COLOR FACTORS IN WHITE AND ALBINO ANIMALS

On this topic only a few statements will be added to those made in the introductory to this paper. Table 11 has been prepared to show the character and origin of the parents, grandparents, etc., of the pairs which supplied the embryos for this study. None of the birds used is capable of producing offspring of a color other than white when mated to any other of the twenty-two birds of the list. None of the offspring of these pairs ever produces retina-choroid pigment. Under the conditions described above all of them can and do form the pigment.

The bearing of this fact on theories of color inheritance which make use of "presence and absence" and "inhibitor" concepts is fairly obvious. Clearly none of these white animals wholly lacks a discreet, segregable, germinal entity, without whose presence the formation of black melanin pigment is impossible. This fact may assist in prescribing limits to the frequent and enormous liberties taken with the "presence and absence" hypothesis. Concerning the wholly hypothetical "inhibitors"—the convenient and ever-present refuge of some geneticists —it may be said that the facts of post-mortem pigment formation seem to suggest one limit beyond which the imagination of their creators

[10] One may recall in this connection that tyrosin was early identified among the products of autolysis by both Jacoby and Salkowski. It is possible that the delay in the post-mortem pigment formation is associated with a delay in the liberation of tyrosin or other chromogen.

TABLE 11

Showing the character and origin of the parents of the embryos used in these studies. Every bird was of white color; all had pigmented irides and non-pigmented choroids. Every pair has produced only white offspring

PARENTS (PAIRS)	SEX	DERIVATION AND RELATIONSHIP	NUMBER OF YOUNG (ALL WHITE) PRODUCED BY THE PAIR	NUMBER OF YOUNG (ALL WHITE) PRODUCED BY PARENTS OF THE PAIR	NUMBER OF YOUNG (ALL WHITE) PRODUCED BY GRANDPARENTS OF THE PAIR.
170	♂	Brother—*St. alba* (7/8)—*risoria* (1/8)			
	♀	Sister—*St. alba* (7/8)—*risoria* (1/8)	64*	77	18†
188a	♂	Brother (from same parents as above)			
	♀	Sister	12	77	18
125y	♂	Brother (= offspring of pair 170 above)			
	♀	Sister	5	64	77
117a	♂	(= Offspring of pair 170 above)	3	64	77
	♀	(= Sister to pair 170 above)		77	18
91	♂	(= Brother to pair 170 above)		77	18
	♀	Extracted white from *alba-risoria-douraca*‡	21		
161d	♂	Brother (= offspring of pair 91 above)			
	♀	Sister	3	21	
2	♂	Brother (= offspring of pair 91 above)			
	♀	Sister	3	21	
161	♂	Brother (sire = brother to pair 170 above, and dam = sister to ♀ of pair 91 above)	9	64	77
	♀	Sister		5	
261	♂	(= Brother to pair 161 above)	5	64	77
	♀	(= Offspring of pair 91 above)		21	
161c	♂	Brother (= offspring of pair 91 above)			
	♀	Sister	1	21	
116m	♂	Nearly pure *St. alba*		6	26
	♀	Extracted white, or "mutant" (?) from blond parents	13		

* None of the embryos or offspring used in these pigment studies is counted in the figures of this column.

† The female grandparent of pair 170 was an extracted white from a crossing of *St. alba* with *St. risoria*.

‡ The parents were ♂ *alba-risoria* and ♀ *alba-douraca-risoria*, of blond and dark color respectively. All of the white young of this and later generations, mated *inter se* or with *St. alba*, have thrown *only* white offspring.

may not go: The regnancy of the color "inhibitor" certainly does not extend beyond death.

The facts of post-mortem pigment formation lend support to the following conclusion of one of us (1), as stated (p. 330) in 1909:

> The specific color of an animal then is an index, not of the presence in the germ from which this animal arose of certain chromogens and specific zymogens, and the absence of a wide series of others; but, this specific color means that a *process* with a wide range of possibilities, *because of a particular physiological state and environmental conditions* has struck this particular equilibrium. One and the same organism has within it all that is necessary to move that equilibrium up or down.

SUMMARY

The post-mortem formation of melanin (black) pigment has been shown to occur in the retina-choroids of white dove embryos of three to twelve days of development. The pigment is not produced in earlier stages and is not as readily produced, possibly not at all, in the full-term or just-hatched embryos. Killing the tissues in $HgCl_2$ does not prevent the development of the pigment. No pigment was formed in the few tested embryos which were previously subjected to a temperature of 100° to 107°C.

The presence of free oxygen is necessary for the production of the pigment. It will form in percentages of oxygen varying between that present in air and 100 per cent. The pigment fails absolutely to form in an atmosphere of CO_2; further, it seems probable that the inner ring of iridial pigment which is present in older living embryos entirely disappears when kept after death in the presence of high percentages of CO_2.

The facts of post-mortem melanin formation have a bearing on current theories of color inheritance and development. They coincide with the view advanced by Riddle in 1909, and present difficulties and limitations to the "presence and absence" hypothesis of color development.

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THE CHEMICAL COMPOSITION OF THE BRAIN OF NORMAL AND ATAXIC(?) PIGEONS

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Received for publication July 13, 1918

Very few data have hitherto been obtained on the chemical composition of the brains of individuals with hereditary mental deficiency or derangement. Fewer still are such data obtained from otherwise healthy, normal individuals whose brains were prepared for analysis at the moment of death. In no case, so far as we are aware, has it been possible to obtain duplicate samples of abnormal brains and to compare them with normals of essentially the same parentage and of similar age. All ofthese advantages are present in the case of the brains of the pigeons here described.

A limited number of chemical studies of human brains, from individuals, dead of various diseases and acute infections, have been made. Still other experiments have been conducted with the object of influencing the chemical composition of the brain of various animals by complete starvation, by underfeeding or by the feeding of specific or particular diets. It does not seem to us that the literature bearing upon these topics requires special treatment here since our data were obtained from brains showing hereditary derangement and particularly since in this material there is reason to believe that something of the condition found at death was continuously present since the early development of the embryo and is, quite probably, not to be wholly accounted for by subsequent degeneration.

MATERIAL AND METHODS

The functional abnormality associated with the brains studied is recognizable and describable as a lack of control of the voluntary movements. This has been shown in different degrees by different individual birds. Occasionally a bird deficient in motor control in early life

becomes normal at a later period and conversely some birds apparently normal in early life may partially lose the control of their voluntary movements in later life. Usually the derangement persists until death.

It is not yet certain by what name this disorder should be called. Even before the present chemical data were obtained, however, there were good reasons for designating the brain as the seat of the disorder. A preliminary description of the origin and inheritance of the disorder has been published by one of us (1); provisionally the disorder has been called ataxia(?).[1] It may here be stated that the more usual manifestations of the disorder are: Nodding of the head or nodding and swaying of the head and neck; unsteady gait; tipping (somersaulting) backwards or forwards; falling on the side; very irregular flight, the bird even flying backwards. The same bird often exhibits two, three or perhaps all of these irregularities. Practically all affected birds are unable and uninclined to sit on a perch, remaining constantly on the ground or on a flat ledge. In the most affected individuals there seem to be no movements whatever of wholly normal coördination; in average cases, however, the disturbances are much increased under excitement, fear or any attempt at increased or more vigorous movement. In a few cases the movements have seemed fairly normal when the bird was at perfect rest. Several of the affected birds have mated and produced young.

The necessary data for age, sex, brain weight, body weight and degree of ataxia are fully given (table 3) for each individual bird and for each group of five birds whose brains were combined into one sample for chemical analysis. The birds were all killed by decapitation and the brain removed immediately, using the following technique: The skull was opened with small bone-forceps so as to expose the convexity of the brain from the fourth ventricle to the olfactory bulbs. An incision was made just posterior to the fourth ventricle, the brain was then lifted up with forceps and the optic nerves cut close to the eyeballs and the olfactory bulbs left attached to the brain. Each brain was quickly weighed and placed in a sufficient quantity of redistilled alcohol to make the final concentration of alcohol about 85 per cent. Five samples were collected in this way. Two of these were controls, one from younger and one from older pigeons; and three samples were formed from brains of birds showing three different degrees of ataxia.

[1] Competent neurologists have undertaken a special study of the brains of these abnormal birds; probably the center or centers of the disorder, or further knowledge of the nature of the disorder, will be disclosed by that study.

In the present paper it is not thought necessary to describe in detail the method (2) used in the determination of the several chemical constituents of the brain further than to say that these brains were allowed to stand in the alcohol for two months, after which a hot alcohol extraction was made, followed by an ether extraction; the brain material was then softened with water after grinding to a fine powder in a mortar. A final 85 per cent alcoholic extraction for fifteen hours completed the extraction. By this procedure the lipoids and water-soluble extractives were separated from the protein residue. The lipoids were then separated from the extractives by precipitation with acid (0.5% HCl and $CHCl_3$), and the sulfur and phosphorus determined in each of these three fractions. Cholesterol was determined in the lipoid fraction.

PRESENTATION OF DATA

Tables 1 and 2 give the analytical results obtained in the normal and ataxic brains. Tables 3 and 4 give general information concerning the nature of the various samples as used for analysis. It is well known that the chemical composition of the brain varies greatly with age; the age of each individual and of each group of individuals is completely given. The sex also is indicated in all cases although it is fairly certain that only slight differences, if any, exist in the percentage composition of the brains of the two sexes.

An examination of the figures of table 1 shows that the group of "most ataxic" pigeons had, among the five groups compared, the highest percentage of water, protein and extractive sulfur; and the lowest percentage of lipoids, phosphatides, and cholesterol. The "more ataxic" group has percentage values which are intermediates of those of the "slightly ataxic" and "most ataxic" groups in practically all of the above-mentioned fractions.

Our own study of these data inclines us to the view that the brains of the ataxic pigeons tend to remain—from a chemical standpoint—in a juvenile or infantile condition. The departures of the various chemical constituents of the ataxic brains from the normal are consistent with the differences known to exist between the brain of the young and the adult. Concerning the relation of age to the chemical composition of the human brain W. Koch (3) concluded as follows:

> Analysis of the brain at different ages shows that with the growth of the brain there is a decrease in the amount of moisture, proteins, extractives and ash; and the cerebrins, lipoids and cholesterin increase. Also there is an increase in the

lipoid sulfur and phosphorus and a decrease in the neutral and inorganic sulfur (=extractive sulfur in tables 1 and 2 of the present paper) and extractive phosphorus.

Koch's published figures on the relation of age to chemical composition of the human brain are placed by us in table 1 to facilitate a comparison between the ataxic and normal pigeon brains in the light of the known changes in composition due to age.

TABLE 1

Constituents of pigeons' brains in percentage of solids

GROUP	I SLIGHTLY ATAXIC (♀♀) 166 DAYS	II MORE ATAXIC (♂♀) 133 DAYS	III MOST ATAXIC (♂♀) 158 DAYS	IV NORMAL (♂♀) 183 DAYS	V NORMAL (♂♂) 106 DAYS	HUMAN BRAIN (13) FOR COMPARISON		
						6 weeks	2 years	19 years
Water in per cent	79.52	79.57	80.25	79.83	79.97	88.78	80.47	76.42
Proteins	49.72	50.86	52.10	50.73	49.99	46.6	40.1	37.1
Lipoids	37.15	36.42	34.87	37.08	36.76	33.1*	47.4*	52.0*
Extractives	13.13	12.72	12.90	12.19	13.24	20.3	12.5	10.9
Phosphatides	22.99	22.39	21.91	23.52	22.93	24.2	25.5	27.3
Sulfatides	6.10	(8.08)	6.26	6.33	6.48	1.6		4.9
Cholesterol	7.37	7.08	6.80	7.50	7.01	1.9	8.7	11.7
Distribution of sulfur								
Protein sulfur	0.527	0.496	0.520	0.483	0.439	62†	59.0	62.0
Lipoid sulfur	0.122	(0.161)	0.128	0.126	0.129	6†	17.0	22.0
Extractive sulfur	0.105	0.104	0.118	0.084	0.099	32†	24.0	15.0
Total sulfur	0.754	(0.761)	0.766	0.694	0.668	0.52	0.58	0.48
Distribution of phosphorus								
Protein phosphorus	0.272	0.289	0.268	0.285	0.295	5‡	6.0	5.0
Lipoid phosphorus	0.892	0.869	0.850	0.912	0.889	54‡	67.0	72.0
Extractive phosphorus	0.341	0.328	0.326	0.304	0.345	41‡	27.0	23.0
Total phosphorus	1.503	1.486	1.444	1.502	1.529	1.72	1.48	1.45

* Total lecithins, kephalins, cerebrins and cholesterin.

† Sulfur in per cent of total sulfur.

‡ Phosphorus in per cent of total phosphorus.

It may be noted that the total phosphorus in the ataxic brains is somewhat less than in the normal. This is contrary to expectation

from the standpoint of the figures given for the relation of age to phosphorus content in the human brain. It will be seen, however, that in the more complete figures given for the rat's brain (appended to table 2) slightly less phosphorus was found in the 10-day than in the 120-day rat. The slightly lower total phosphorus values found in the ataxics as compared with normals is therefore possibly not valid evidence against the juvenile character of the ataxic brains.

Details of the distribution of sulfur and phosphorus calculated in per cent of total sulfur and total phosphorus are given in table 2. To table 2 are added for comparison the same fractions obtained from albino rats of various ages. These figures (thus calculated) obtained on the rat indicate: *a*, Protein sulfur is present in smallest amounts in the youngest stages. The "more ataxic" group of brains seems to show this smallest proportion of protein sulfur. *b*, Lipoid sulfur is similarly present in smallest amounts in the youngest stages of the rat. The "slightly ataxic" and "most ataxic" groups of pigeons show lower values than either of the two normal groups. *c*, Extractive sulfur is present in greatest amount in the youngest stages of the rat. It is present in greatest amount in the "most ataxic" pigeons. *d*, Protein phosphorus is apparently less in rats of twenty days than in either older or younger stages. The five groups of pigeons brains show no consistency in this figure, either in reference to age or to affected and unaffected brains. *e*, Lipoid phosphorus is lowest in youngest rats. It is lowest in the much younger normal pigeons but plainly lower in the three groups of ataxics than in the older normals. *f*, Extractive phosphorus is found in greatest relative proportion in the youngest stages of the rat. All three groups of "ataxic" pigeons show higher values than do the older group of normals, but values in close agreement with those of the much younger normals.

DISCUSSION

There are several questions which naturally arise in connection with an examination and interpretation of the analytical results obtained on these pigeon brains. We have sought to supply data probably useful as giving answer to some of those questions, in the form of tables.

Tables 3 and 4 show that the body weight and brain weight of the males used were greater than those of the females and that the average age of the males was less by fifteen days. There is little of real immaturity, however, in either group, the youngest birds (table 4) of either sex showing brain weights nearly or quite equal to the average for their

TABLE 2

Distribution of sulfur and phosphorus in percentage of total sulfur and total phosphorus

GROUP	I	II	III	IV	V
Protein sulfur	69.88	65.12	67.93	69.64	65.80
Lipoid sulfur	16.18	(21.22)	16.70	18.24	19.40
Extractive sulfur	13.94	13.66	15.37	12.12	14.80
Total sulfur (in per cent of solids)	0.754	(0.761)	0.766	0.694	0.668
Protein phosphorus	18.09	19.46	18.52	19.00	19.28
Lipoid phosphorus	59.25	58.44	58.88	60.77	58.17
Extractive phosphorus	22.66	22.10	22.60	20.33	22.55
Total phosphorus (in per cent of solids)	1.503	1.486	1.444	1.502	1.529

The same chemical groups in albino rat of different ages for comparison (14)

GROUP	AGE IN DAYS				
	1	10	20	40	120
Protein sulfur*	31.10	48.60	57.50	65.10	61.20
	30.00	44.20	55.30	62.40	62.40
Lipoid sulfur	3.20	6.10	6.70	9.20	12.80
	2.80	6.70	7.50	10.10	12.50
Extractive sulfur	65.70	49.20	35.80	25.70	26.00
	67.20	49.70	37.20	27.50	25.10
Total sulfur (in per cent of solids)	0.96	0.72	0.69	0.58	0.55
	1.04	0.83	0.70	0.52	0.57
Protein phosphorus	13.30	13.00	6.00	9.90	7.40
		13.90	5.80	7.50	7.30
Lipoid phosphorus	33.20	33.80	52.20	56.10	65.80
	33.00	36.10	53.50	58.50	62.30
Extractive phosphorus	53.50	53.20	41.80	34.00	26.80
	53.60	50.00	40.70	34.00	30.40
Total phosphorus (in per cent of solids)	1.82	1.28	1.66	1.55	1.40
	1.92	1.48	1.67	1.50	1.44

* Duplicate analyses throughout this part of the table.

TABLE 3

Data on the age, sex, brain weight, body weight and degree of ataxia of the five groups of pigeons which supplied samples for analysis

NUMBER OF SAMPLE	DESCRIPTION OF GROUP		SEX AND NUMBER	AGE	BRAIN WEIGHT	BODY WEIGHT
				days	*grams*	*grams*
I	Slightly ataxic	Females (5)	♀M357	125	1.780	332
			♀M355	129	1.831	309
			♀K143	143	1.605	297
			♀K175	183	1.835	308
			♀K136	249	1.867	325
Average of sample				166	1.784	314
II	More ataxic	Females (2)	♀K249	69	1.811	292
			♀M315	127	1.813	336
		Average		98	1.812	314
		Males (3)	♂M3450	124	1.943	340
			♂K181	161	1.978	331
			♂K180	182	1.955	358
		Average		156	1.959	343
Average of sample				133	1.900	331
III	Most ataxic	Females (3)	♀K242	83	1.800	309
			♀K220	158	1.670	303
			♀K182	185	1.736	340
		Average		142	1.735	317
		Males (2)	♂K166	172	1.842	350
			♂K170	191	1.897	326
		Average		182	1.870	338
Average of sample				158	1.789	326
IV	Normal (older)	Females (3)	♀K197	181	1.743	362
			♀K124	186	1.782	349
			♀K117	216	1.935	349
		Average		194	1.82	353
		Males (2)	♂K195	158	2.082	367
			♂K163	173	1.855	382
		Average		166	1.969	374
Average of sample				183	1.879	362

TABLE 3—*Continued*

NUMBER OF SAMPLE	DESCRIPTION OF GROUP		SEX AND NUMBER	AGE	BRAIN WEIGHT	BODY WEIGHT
				days	*grams*	*grams*
V	Normal (younger)	Males (5)	♂K264	69	1.809	324
			♂K277	83	1.900	357
			♂K260	109	1.940	398
			♂M301	123	2.065	342
			♂K222	144	1.895	380
Average of sample				106	1.922	360

* The age of each bird includes the whole of its incubation period.

TABLE 4

Males and females whose brains were analyzed listed separately and in order of increasing age

FEMALES				MALES			
Number and description of birds	Age	Brain weight	Body weight	Number and description of birds	Age	Brain weight	Body weight
	days	*gms.*	*gms.*		*days*	*gms.*	*gms.*
K249 (more ataxic)	69	1.811	292	K264 (normal)	69	1.809	324
K242 (most ataxic)	83	1.800	309	K277 (normal)	83	1.900	357
M357 (slightly ataxic)	125	1.780	332	K260 (normal)	109	1.940	398
M315 (more ataxic)	127	1.813	336	M301 (normal)	123	2.065	342
M355 (slightly ataxic)	129	1.831	309	M3450 (more ataxic)	124	1.943	340
K143 (slightly ataxic)	143	1.605	297	K222 (normal)	144	1.895	380
K220 (most ataxic)	158	1.670	303	K195 (normal)	158	2.082	367
K197 (normal)	181	1.743	362	K181 (more ataxic)	161	1.978	331
K175 (slightly ataxic)	183	1.835	308	K166 (most ataxic)	172	1.842	350
K182 (most ataxic)	185	1.736	340	K163 (normal)	173	1.855	382
K124 (normal)	186	1.782	349	K180 (more ataxic)	182	1.955	358
K117 (normal)	216	1.935	349	K170 (most ataxic)	191	1.897	326
K136 (slightly ataxic)	249	1.867	325				
Average	156	1.785	324	Average	141	1.930	355

sex. The additional fact may be stated here that our determination of the body weights of several much older birds of this strain, normals and ataxics, indicates an average body weight scarcely, if any, higher than that shown in the birds which supplied the samples for analysis. It is not yet definitely known whether the brain weight of these birds increases beyond the age represented by the birds used in our analyses;

the fact referred to above, however, concerning the approximate average brain size of the youngest birds selected, would argue against any later considerable increase in brain weight.

A summary comparison of some of our results with those of earlier investigations on the pigeon's brain is given in table 5. Lukjanow (4), who seems to have used a smaller variety or species of pigeon than ours, published no data as to the variety or the age of the birds studied by him. Funk (5) also failed to note the age of the pigeons used in his studies. The brain weights and body weights of his birds indicate that in these respects the birds used were fairly comparable with those used by us.

The questions just raised concerning brain size and body size perhaps deserve a short additional statement. Our ataxic birds were somewhat undersized as compared with the normals although not markedly so; and the fact that the older ataxics were larger than the younger ataxics, essentially in the order of age, makes it reasonably clear that these birds had grown continuously although more slowly than the normals. It is possible that the "most ataxic" birds, because of the extreme awkwardness of movement, sometimes failed to obtain as much food as they wanted. On this point it may be noted that Hatai (6) has shown that temporary underfeeding, in the albino rat, leaves unchanged the relation of brain weight to body weight. Donaldson (7) observed a 2.5 per cent increase in the relative brain weight of rats given special opportunities for voluntary exercise. It is probable that the ataxic pigeons do not exercise as much as the normals and part of the discrepancy in brain weight of these ataxics may be thus explained.

The studies of Mott (8), Carlyll and Mott (9) and S. A. Mann (10), on the brains of children dead of amaurotic dementia (Tay-Sach's disease) are of much interest in the present consideration of the chemical composition of the brain in hereditary mental disease. Attention can here be directed, however, only to some seemingly significant agreements in the two series of data and to the nature of the interpretations offered by the above-mentioned investigators for the observed chemical composition of the brain in amaurotic dementia.

The results of Mott and of Mann[2] indicate higher moisture values in this disease. Notably less lipoid phosphorus and sulfur with a corresponding increase of the extractive forms were found. The values in the affected brains for total extractives, cerebrosides and cholesterol seem unchanged

[2] Mann's analyses were carried out by the method of W. Koch.

TABLE 5

A comparison of present (normal and ataxic) with earlier data obtained from investigations on the pigeon's brain

DESCRIPTION	BODY WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	INVESTIGATOR
Normal				
	grams	*grams*	*per cent*	
10♂	292.6	1.908	79.94	Lukjanow
10♀	256.6	1.850	80.37	
Average	274.6	1.879	80.16	
Average	320	1.80	78.62	Funk
IV {3♀ 2♂}	362	1.879	79.83	Koch and Riddle
V 5♂	360	1.922	79.97	
Average	361	1.900	79.90	
Experimental (and abnormal)				
Starved for:	*Init. and fin.*			Lukjanow
154 hours, ♂	299 to 189	1.881	79.73	
151 hours, ♀	274 to 190	1.887	79.82	
Average	287 to 190	1.884	79.78	
Starved till dead ♂	288 to 159		82.44	
Starved till dead ♀	242 to 149		80.79	
Average	265 to 154		81.62	
Polyneuritis	300 to 232	1.75	79.42	Funk
Cured of polyneuritis	314 to 259	1.82	78.50	
Underfed	352 to 269	1.98	78.14	
Average	322 to 253	1.85	78.69	
I. Slightly ataxic 5♀	314	1.784	79.52	Koch and Riddle
II. More ataxic {2♀ 3♂}	331	1.900	79.57	
III. Most ataxic {3♀ 2♂}	338	1.789	80.25	
Average	328	1.824	79.78	

or inconsistently changed. A very pronounced change was found in the relation of nucleo-proteins to simple proteins. The former were much decreased. This result also is paralleled in the pigeon brains, as will be seen from the following calculation[3] of the amount of nucleo-protein in the five groups of pigeon brains: I, 5.94; II, 6.38; III, 5.78; IV, 6.30; V, 6.51. Perhaps the most noticeable difference in the affected human and pigeon brains is that total sulfur and total phosphorus were both found by Mann to be decreased in the human, while in the pigeon we find the total phosphorus decreased and the total sulfur increased. The chemical variations from the normal as observed in the ataxic pigeon brains and in human amaurotic dementia are apparently alike in kind.

The above-mentioned workers did not in general interpret their results in terms of infantilism or under-development. The following quotations from their publications, which are also concerned with clinical and histological studies, are given as indicative of their interpretations and conclusions:

Mott (1905) states that

> The profound affection in the physiological functions of the central nervous system which characterizes this disease may be especially associated with a bio-chemical change in the metabolism of the nucleus. The cause of this regressive metabolic metamorphosis may be an inherent lack of specific energy, racial or familial, of the neurones and possibly some hitherto undiscovered bio-chemical alteration of the blood or lymph. The existence of the neuro-fibrils, which may be regarded as the conductile structure of the neurones, which clinical facts tend to show had ceased to function, would support the view either that the Nissl substance which had disappeared was itself an important agent in neural function, or that it was an antecedent of a substance at the synapses. The increase of neuroglia fibril substance to an abnormal degree in case I, so that the brain weighed heavier than that of a normal adult, accounts for the large amount of simple proteins and the great diminution of nucleo-proteins.

Carlyll and Mott later (1911) examined seven cases of Tay-Sach's disease, two of which were analyzed chemically by Mann. These authors conclude that the cause of this disease is "probably a failure in the germinal determinants of the nervous system peculiar to the Jewish race," and set forth the hypothesis that

> It may be due to a failure in the nuclear material of the neurone to build up the nucleo-protein Nissl substance out of lipoid substances contained in the cyto-

[3] Obtained from protein phosphorus by factor (175.4) as worked out by Levene (11).

plasm which first has to be decomposed by nuclear ferments. . . . . The chemical analysis does not throw much light upon the question; the diminution of the lipoid form of phosphorus and sulfur is probably due to the diminution of myelin owing to the failure of development[4] of the myelinated fibers. The corresponding increase of extractive forms of phosphorus and sulfur may be possibly due to a breaking down of the more complex to simpler forms of lipoids.

Mann states that some (not all) of the changes observed "may be explained as the result of nutritive changes."

We believe that the care used in the preparation of the five groups of brains and the degree of accuracy of the methods of analysis justify our conclusions concerning the differences found in the analytical results. We hope, however, to be able further to test and amplify the results reported here by an investigation in which the cerebellum is analyzed apart from the remainder of the encephalon.

The birds used are all inbred for two to four generations. The original ataxic female—which arose under conditions known in pigeons to produce *weakened* germs,—is a common ancestor of them all (normals and ataxics). The stock is mongrel common pigeon but nearly all of the birds are three-fourths homer (*C. tabellaria*) and one-fourth mongrel; a few are about one-fourth rock-pigeon (*C. livia*). Since the derangement (ataxia?) characteristic of these birds arose as a wide variation (mutation?) under conditions known (12) to favor or induce the production of females, individuals of shorter life-term, and less vigor, it is of added interest that the chemical composition of these ataxic brains indicate a retardation or restriction of the development of an important part[5] of the central nervous system.

SUMMARY

The brains of birds which have very little control of the voluntary movements (ataxia?) have been analyzed. The data thus obtained are compared with similar data obtained on the brains of normal birds of the same parentage.

The derangement first appeared (mutation?) under conditions known to lead to weakness in the offspring. The disorder is exhibited in all degrees and has been inherited undiminished to the fifth generation.

[4] This statement is, of course, somewhat similar in nature to the interpretation applied generally by the authors of the present paper.

[5] It is, of course, not necessarily to be inferred that the whole of the encephalon is chemically under-differentiated. Possibly some particular localized areas are solely or chiefly involved.

Usually it is shown from the earliest age of the bird and is probably present throughout the whole development of the bird.

The brains of the affected individuals show increased values for moisture, protein and extractive sulfur; decreased values for lipoids, phosphatides and cholesterol. In general the less ataxic individuals show values intermediate to those of the normals and most ataxics. The distribution of sulfur and phosphorus in the various chemical fractions was determined.

The results of the analyses of the affected brains are interpreted as suggesting a chemical under-differentiation or immaturity of these brains. The brains of affected birds of approximately mature age are chemically more like the brain at earlier stages of development.

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A NEW FORM OF AEROTONOMETER

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Received for publication July 19, 1918

In the series of experiments in which we undertook to investigate the possibilities of the lung as an aerotonometer, we used in the first instance, for purposes of comparison, one of Krogh's aerotonometers (1). Important difficulties were encountered in using this tonometer. First, the time taken to reach equilibrium. Under favorable conditions it takes at least twenty minutes and usually longer owing to the fact that the blood tends to run in streaks down the tube, thus diminishing the surface available for diffusion. Second, only a small quantity of gas is obtainable for analysis and since it is desirable that all analyses be made in duplicate, this is a serious drawback.

The following is a description of a new type of aerotonometer designed by the late Prof. T. G. Brodie, which has been found to be very satisfactory. It consists of two horizontally placed glass cylinders, one inside the other. The outer tube is 236 mm. in length and 27 mm. in outside diameter. At either end the tube is flanged to 30 mm.; the inside diameter is 23.5 mm. The inner tube is supported by a ground glass joint to one extremity of the outside tube. Its length is 223 mm. and outside diameter 18 mm. It is closed at its free end and the other end projects 20 mm. from the outside tube to afford attachment to a wheel which is revolved by belting from the shafting. The outer tube rotates on a ground glass surface, about a rubber cork surrounded by a shell of ground glass. This cork fits into the other flanged end of the outside tube. Through the cork pass three tubes; a fine silver tube, *A*, to bring the blood into the tonometer; a thick glass tube, *V*, which carries the blood back, and another fine silver tube, *X*, from which air samples may be taken. The blood enters through the fine silver tube, situated lowest in the cork. This tube carries the blood to the far end of the apparatus. The blood is returned by the middle thick glass tube which dips down to the bottom of the tube immediately on its entrance. The space between the two cylinders is kept half full of

blood so that the upper half is filled with gas. The fine silver tube entering fom the top of the cork is in communication with this gas. On its exit the tube bifurcates, one limb leading to a manometer, M, and from the other limb samples of this gas may be extracted for analysis.

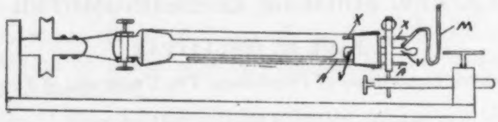

Fig. 1

The volume between the two cylinders is about 34 cc. When half full of blood this leaves 15 cc. of gas, an ample volume for several analyses.

In estimating the relative value of different tonometers, great stress has been laid on the "specific surface" which, as defined by Krogh (2), is the ratio of the volume of gas in cubic centimeters to the surface available for diffusion in square centimeters.

The specific surface of this tonometer is about 7.3, as shown in the following calculation.

$$\text{Specific surface } \frac{\text{Area}}{\text{Volume}} \frac{\frac{1}{2}(2\pi\gamma l + 2\pi\gamma' l)}{\frac{1}{2}(2\pi\gamma^2 l - \pi\gamma'^2 l)} = \frac{2\pi l(\gamma + \gamma')}{\pi l(\gamma + \gamma')(\gamma - \gamma')} =$$

$$\frac{2}{\gamma - \gamma'} = \frac{2}{0.275} = 7.3.$$

where l = length of inner cylinder.

γ = radius of inner surface of outer cylinder = 1.75.

γ' = radius of outer surface of inner cylinder = 0.9.

The thickness of the films of blood on both the inner and outer tube would appreciably diminish the volume of gas and thus increase the relation of area to volume so that the apparatus has probably an actual specific surface of about 10.

Compared with older tonometers on this basis no special advantage has been obtained. The earliest used, Pflüger's, had a specific surface of 3.3 while the latest pattern of Krogh's (leaving aside his micromonometer) has a specific surface of about 30. But it must be here pointed out that the rapidity of diffusion depends not alone on the specific surface, but also on the rate or renewal of that surface.

With each rotation of the tonometer a new surface of blood is presented for diffusion whereby the exchange of gases is greatly facilitated. It would seem desirable then, to employ as a unit in comparing aerotonometers the "specific surface per minute;" that is to say, the surface in square centimeters divided by the volume of gas in cubic centimeters and multiplied by the number of times that surface is renewed per minute.

The tonometer was usually revolving at the rate of 30 revolutions per minute so that the "specific surface per minute" of this apparatus may be put at 300.

It is not easy to calculate the specific surface per minute of Krogh's apparatus but it would probably reach a value of 100, as indicated in the following estimation.

Air space 5–15 cc. say 10.

Rate of flow of fluid 15–44 cc. minutes say 30.

Specific surface 20–33 say 30.

Specific surface per minute—$\frac{30}{10} \times 30\text{-}90$

To ascertain the equilibrium time of the tonometer a series of experiments was undertaken in which a quantity of defibrinated blood was circulated through the apparatus and samples of gas extracted at different times. The perfusion apparatus used was the same as that employed in the experiments on the excised lung (3).

The following experiments will serve to indicate the accuracy of the method and the time taken for equilibrium to be attained.

February 10, 1912. Dog's blood defibrinated. Barometer 755 mm. Hg., Temperature 18°C.

Experimental conditions and intervals in minutes	Pressure of gas in mm. Hg in tonometer	Samples of gas from tonometer					
		CO_2			O_2		
		Analysis	Mean	Tension	Analysis	Mean	Tension
		per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm*
Tonometer filled with room-air							
0 (circulation established)	−0.0						
10	−0.5	0.19	0.15	1.1	19.83	19.78	146.3
		0.12			19.73		
15	−1.0	0.20	0.16	1.2	19.70	19.67	145.4
		0.12			19.64		

February 10, 1912.—Continued.

EXPERIMENTAL CONDITIONS AND INTERVALS IN MINUTES	PRESSURE OF GAS IN MM. HG IN TONOMETER	SAMPLES OF GAS FROM TONOMETER					
		CO_2			O_2		
		Analysis	Mean	Tension	Analysis	Mean	Tension
		per cent	*per cent*	*mm.*	*pre cent*	*per cent*	*mm.*
Tonometer filled with expired air							
0	−0.0						
10	−1.0	0.53 0.44	0.48	3.5	19.44 19.33	19.38	143.2
15	−0.5	0.44 0.40	0.42	3.1	19.20 19.21	19.20	142.2

February 13, 1912. 140 cc. of defibrinated dog's blood. Barometer 765.8 mm. Hg.

EXPERIMENTAL CONDITIONS IN THE CIRCULATION AND INTERVALS IN MINUTES	TEMPERATURE	PRESSURE OF GAS IN MM. HG IN TONOMETER	SAMPLES OF GAS FROM TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
	deg. C.		*per cent*	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
10	16.8	−2.5	2.85 2.92	2.89	21.7	18.00 17.89	17.94	134.5
15	18.0	−4.0	3.27 3.18	3.22	24.0	17.46 17.54	17.50	130.5
Tonometer filled with expired air								
10	19.7	−4.0	3.65 3.66	3.65	27.2	17.77 17.66	17.70	132.1
15	20.5	−5.5	3.75 3.70	3.72	27.6	17.59 17.64	17.62	130.8
Tonometer filled with expired air								
5	21.8	−1.7	3.90 3.92	3.91	29.1	17.45 17.48	17.47	130.1
10	21.8	−2.5	3.89 3.84	3.87	28.8	16.66 16.64	16.63	123.7

March 20, 1912. 135 cc. of defibrinated dog's blood in the system. Rate of flow 70 cc./minutes. Circulation time once every 2 minutes. Barometer 755 mm. Hg.

EXPERIMENTAL CONDITION AND INTERVAL IN MINUTES	TEMPERATURE	PRESSURE OF GAS IN TONOMETER	SAMPLES OF GAS FROM TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
		deg. C.	per cent	per cent	mm.	per cent	per cent	mm.
Expired air into tonometer								
15	22.8	0.5	1.06 1.12	1.09	8.0	17.79 17.83	17.81	130.9
20	22.8	0	1.06 1.01	1.04	7.6	17.78 17.77	17.78	130.7
Expired air into tonometer								
15	21.0	0.5	1.09 1.17	1.13	8.3	16.62 16.78	16.70	123.1
20	20.8		1.34 1.36	1.35	9.9	16.81 16.93	16.87	124.4

April 9, 1912. 225 cc. of defibrinated blood. Rate of flow 100 cc./minutes. Barometer 755 mm.

EXPERIMENTAL CONDITION AND INTERVAL IN MINUTES	PRESSURE OF GAS IN TONOMETER	TEMPERATURE	SAMPLES OF GAS TAKEN FROM TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
	deg. C.	deg. C.	per cent	per cent	mm	per cent	per cent	mm.
0 (circulation established)								
15	1.0	21.	1.19 1.15	1.17	8.6	16.56 16.41	16.48	121.3
20	1.5	21.	1.12 1.20	1.16	8.5	16.35 16.39	16.37	120.1
Expired air into tonometer								
20	−1.0	21.8	1.02 0.98	1.00	7.3	17.43 17.50	17.46	128.3
25	−1.5	21.2	1.06 0.91	0.99	7.3	17.39 17.36	17.37	127.6
Expired air into tonometer								
15	−1.0	20.6	1.08 1.10	1.09	8.0	15.46 15.53	15.50	114.0
20	−1.5	20.8	1.01 0.98	1.00	7.3	15.57 15.60	15.59	114.6

DISCUSSION

In the experiment of February 10, the sample of gas taken after the blood had been in contact with the gas in the tonometer for 15 minutes showed the same tension as the sample taken after 10 minutes. In the one of February 13, the last set of samples which was taken 5 and 10 minutes after air was expired into the tonometer, differed to an extent greater than could be accounted for by experimental error. The factor of the rapidity with which the blood is circulating would modify the results obtained and in the experiment of April 9, this point was taken into account by measuring the rate of blood flow. The figures obtained in this instance show that when a good rate of flow is obtained, equilibrium is reached within 15 minutes, and probably in 10 minutes as shown in the experiment of February 10.

CONCLUSIONS

A new type of aerotonometer is described in which equilibrium between blood and air was reached certainly in fifteen minutes, and with a good rate of flow in ten minutes.

It possesses the further advantage of providing abundant gas for duplicate analyses.

The credit of designing this apparatus belongs exclusively to the late Prof. T. G. Brodie; my small contribution consisted in carrying out the experiments and the responsibility for the accuracy of the gas analyses rests with me.

In some experiments in which the rate of flow through the system was not observed, equilibrium was not reached in 10 minutes. Several experiments were then undertaken in which the rate of flow was particularly attended to.

The rate of flow was estimated by diverting the flow for a few seconds from the rubber tube where it leaves the tonometer into a graduated cylinder. The time was determined by a stop watch. Several readings were taken, the blood withdrawn being restored to the circulation after each estimation through the funnel. (See description of the artificial circulation in the article "The lung as an aerotonometer.")

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THE LUNG AS AN AEROTONOMETER

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Received for publication July 19, 1918

In contemplating some experiments which required that the gaseous tension of the blood in a living animal be quickly and accurately determined, the need of an efficient aerotonometer was encountered.

From a priori considerations an excised lung would seem to afford an ideal aerotonometer on the supposition that the alveolar surface of an excised lung, when perfused, acted solely as a physical structure. Here the blood is spread out in an exceedingly fine layer, approximating to the thickness of one blood corpuscle and exposed to the process of diffusion with the air in the alveoli. The area of surface of exposure of the blood, measured in square centimeters is more than two hundred times the volume of air enclosed in the alveoli, measured in cubic centimeters. Moreover in a perfused lung this surface of blood is rapidly renewed.

To test this hypothesis the following experiments were performed. *In the first series of experiments* the inflated excised lung was perfused through its vessels with defibrinated blood and at various intervals a sample of gas was withdrawn from the trachea for analysis. If successive samples of gas showed the same composition, the gaseous pressure remaining constant, it would indicate that equilibrium between the gases in the blood and the gases in the alveoli had been attained.

An inflated human lung has a capacity of about 3700 cc. and a surface available for exchange of gases of about 90 sq. m. This would mean a specific surface (area of exchange surface in square centimeters divided by volume of gas enclosed in cubic centimeters) of about 240.

METHOD

In these experiments a dog was bled to death; the blood was whipped and filtered through glass wool. The thorax was opened and cannulae inserted into the pulmonary artery, before its bifurcation and into the left auricular appendix. A strong ligature was tied around the heart so as to close the auricular-ventricular openings and thus the systemic

circulation was excluded. A glass tube was inserted into the trachea and was connected by rubber tubing with a mercury receiver. By raising and lowering this receiver the air in the lung alveoli was mixed with the dead space air. Gas samples could be extracted from a side tube.

Figure 1 is a diagram of the artificial circulation through which the defibrinated blood was perfused, the blood being introduced through the funnel *X*, which is closed by a weighted mercury stopper. About 250 cc. of blood are required to fill the system of tubes and the blood vessels of the lungs. A mean air pressure is transmitted to a rubber bag, *B*, which acts upon the blood contained in the arterial glass reservoir, *R1*. This air pressure is obtained by attaching a tube from the

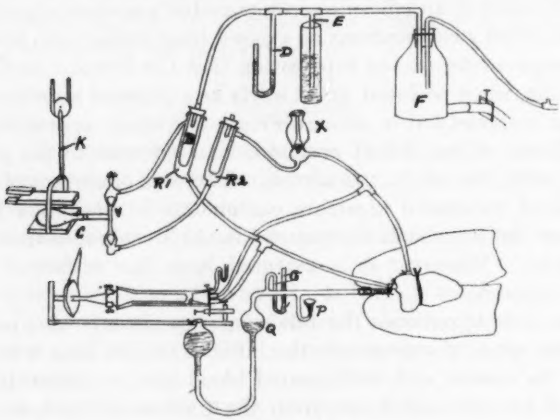

Fig. 1.

water tap and directing it into a large 10 liter jar, *F*, tightly corked. The water runs away from the large orifice at the bottom of the jar, and this exit is provided with rubber tubing and a screw clamp. By tightening this screw the rate of outflow may be lessened and thus the pressure of air in the jar above the water is increased. On the path of the tubing between the jar and the arterial reservoir are two side tubes. One, *D*, serves as a manometer; the other side tube, *E*, dips into the long cylinder containing mercury and by altering its depth in the mercury the pressure in the system is regulated. The blood is driven into the arterial reservoir, *R1*, by the pump, *C*, a rubber bag alternately compressed and relaxed by a crank movement, *K*, operated by a motor. The pump is provided with a ground glass valve, *V*, of well-known de-

sign, which insures that the direction of flow shall be toward the arterial reservoir. From the arterial reservoir the blood is driven through rubber tubing into the pulmonary artery and collected again by the cannula in the left auricular appendix and sent into another, the venous reservoir, *R2*. The venous reservoir is a thick glass tube exactly similar to the arterial one, provided with an elastic bag by means of which the venous pressure may be altered by blowing into the tube inserted into the cork which closes the open end. The blood from the lungs enters the venous reservoir at the corked end of the tube and leaves the reservoir from the opening at the opposite end. From this point the blood is returned to the valve by tubing and the circulation is completed. The return flow is assisted by gravity, the valve being placed lower than the venous reservoir. In the first series of experiments the lung was perfused with defibrinated dog's blood.

The lungs are inflated and the trachea is attached by rubber tubing to the mercury receiver, *Q*. Before taking the sample, the air in the trachea, rubber tubing and mercury bulb (dead space air) is thoroughly mixed by raising and lowering the mercury receiver. A manometer, *G*, is also attached to this tube which gives the pressure of air in the lungs at the time sample is taken. In some cases the entire mechanism was enclosed in a thermostat and the experiment conducted at about body temperature. Air samples are withdrawn through the mercury seal, *P*, the tip of the gas-sample pipette fitting into a small piece of rubber tubing at the bottom of the little cup attached to the T-piece, which is filled with mercury. When a sample is taken it is sealed off with mercury and may be analyzed at leisure. Analyses in duplicate are made and from them the gaseous tensions of the gases in the lung are estimated. For example, supposing the barometer stands at 757 mm., the temperature of the blood in the system is 36°C., the manometer attached to the tube to the trachea shows a pressure of 2 mm. An analysis of a sample taken shows:

$$CO_2 = 3.24 \text{ per cent}$$
$$O_2 = 15.75 \text{ per cent}$$

then the total pressure of the gases in the lung = 757.0 − 2 = 755.0 mm.
deducting pressure of aqueous vapour at 36°C. = 44.2 mm.
then the pressure due to CO_2 plus O_2 plus N_2 = 710.8 mm.

$$\text{therefore tension of } CO_2 = \frac{3.24}{100} \times 710.8 = 23 \text{ mm. Hg.}$$

$$\text{tension of } O_2 = \frac{15.75}{100} \times 710.8 = 112 \text{ mm. Hg.}$$

and the nitrogen is obtained by deducting the sum of these two from the total pressure less aqueous tension. Samples are taken at 1, 2, 3, 4 and 5 minutes. When successive samples show the same tension, then it is assumed that equilibrium has been reached.

RESULTS

The following figures will show some of the results obtained.

March, 1911. Blood obtained from dog. Thorax in thermostat at 36°C. Barometer 755 mm. Hg.

TIME	OBSERVATIONS	INTERVAL IN MINUTES	GAS—SAMPLES FROM LUNGS: CO_2 Analysis	CO_2 Mean	CO_2 Tension	O_2 Analysis	O_2 Mean	O_2 Tension
			per cent	*per cent*	*mm. Hg.*	*per cent*	*per cent*	*mm. Hg.*
10.56	Commenced mixing alveolar air with air in dead space							
10.59	Sample 1 withdrawn......	3	3.17 3.31	3.24	23	15.88 15.63	15.75	112
11.05	Sample 2..................	9	3.19 3.11	3.15	22	15.32 15.57	15.45	110
11.10	Sample 3..................	14	2.89 2.88	2.88	20	15.23 14.94	15.08	107

March 7, 1911. Blood obtained from dog ♀ 15 kgm. Thermostat 36°C. Barometer 755 mm.

TIME	OBSERVATIONS	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNG: CO_2 Analysis	CO_2 Mean	CO_2 Tension	O_2 Analysis	O_2 Mean	O_2 Tension
			per cent	*per cent*	*mm. Hg.*	*per cent*	*per cent*	*mm. Hg.*
12.29	Circulation established							
12.40	Commenced mixing gases							
12.41½	Sample 1..........................	1½	4.53 4.32	4.42	31	13.89 14.09	13.99	99
12.46	Sample 2..........................	6	4.56 4.64	4.60	33	13.63 13.68	13.65	97
12.52	Sample 3..........................	12	4.99 4.90	4.95	35	13.31 13.25	13.28	94
1.08	Sample 4..........................	38	5.42 5.57	5.48	39	12.58 12.38	12.48	91

March 10, 1911. Blood from dog ♂ temperature 30°C. Barometer 755 mm. Hg.

DATE	OBSERVATIONS	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNGS					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	*mm. Hg.*	*per cent*	*per cent*	*mm. Hg.*
2.56	Circulation established							
3.00	Sample 1........................	4	1.83	1.85	13	16.25	16.08	120
			1.89			15.92		
3.02½	Sample 2........................	6½	1.47	1.78	13	16.54	16.38	120
			2.10			16.22		
3.32	Sample 3........................	36	1.85	1.84	13	14.89	14.83	108
			1.83			14.77		

June 13, 1911. 225 cc. of blood from dog 14 kgm. Barometer 755 mm. Hg.

TIME	OBSERVATIONS	LUNG PRESSURE	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNG					
					CO_2			O_2		
					Analysis	Mean	Tension	Analysis	Mean	Tension
		mm. Hg.	*deg. C.*		*per cent*	*per cent*	*mm. Hg.*	*per cent*	*per cent*	*mm. Hg.*
12.00	Circulation established									
12.10	Sample 1............	9	28.5	10	3.87	3.86	28.4	16.00	15.94	117
					3.85			15.87		
12.30	Sample 2............	8	30.0	30	4.02	4.00	29.3	14.99	14.93	109
					3.98			14.86		
1.04	Sample 3............	6	32.5	64	4.07	4.09	29.6	13.77	13.71	99
					4.12			13.65		
1.35	Sample 4............	5	34.5	95	4.18	4.21	30.3	12.27	12.29	88
					4.25			12.31		
2.17	Sample 5............	5	34.0	137	4.16	4.10	29.5	9.92	9.98	72
					4.05			10.04		

From these experiments it will be seen that samples taken within ten minutes of each other agree fairly well whereas a sample taken after an interval of twenty minutes or thirty minutes after a given sample shows an obvious decrease in oxygen tension. This may be accounted for by the oxygen metabolism of the living elements of the blood. For example in the experiment of June 13, the oxygen tension fell from 117

mm. to 72 mm. in 127 minutes. At the CO_2 tension of 30 mm. this corresponds to a fall in oxygen content from 98 per cent saturation to 94 per cent saturation (1). There were 225 cc. of blood in the apparatus. Assuming that 100 cc. of dog's blood when saturated contain 18 cc. of oxygen, and neglecting the amount physically dissolved in the plasma, this means an oxygen consumption of 0.000026 cc./gm./min. Krogh's figures (2) for the oxygen-metabolism of rabbit's blood (hirudinized) vary from 0.00046–0.00075 cc./gm./min. at body temperature.

Another experiment in which this feature was especially noted is here given.

April 10, 1911. 225 cc. of blood (dog). Temperature 31°C. Barometer 755 mm. Hg.

	Time in minutes	Air samples from lung					
		CO_2			O_2		
		Analysis	Mean	Tension	Analysis	Mean	Tension
		per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
Sample 1, 7 mm.	0	3.36	3.37	24.6	14.36	14.30	104.5
		3.39			14.24		
Sample 4, 7 mm.	113	3.54	3.55	25.9	7.84	7.50	54.8
		3.55			7.16		

At a temperature 31°C. $\alpha = 0.0258$ (α = coefficient of absorption of oxygen dissolved in water).

At 104 mm. of O_2 (CO_2 tension 25 mm.) 225 cc. of blood contain

$$\frac{25.8 \times \frac{14.30}{100} + 180 \times \frac{98}{100} \times 225}{1000} = 40.52 \text{ cc. of } O_2$$

At 55 mm. of O_2 (CO_2 tension 26 mm.) 225 cc. of blood contain

$$\frac{25.8 \times \frac{7.50}{100} + 180 \times \frac{85}{100} \times 225}{1000} = 34.85 \text{ cc.}$$

225 cc. of blood use up 5.67 cc. of O_2 in 113 minutes—about 0.00022 cc./gm./min.

With increase in temperature of about 10°C. the oxygen consumption increased about tenfold. In all cases the fall in oxygen tension can be accounted for by the oxygen metabolism of the living elements of the blood.

In the second set of experiments one of Krogh's aerotonometers was inserted into the circulation in parallel with the lung. This apparatus takes from twenty to thirty minutes to attain equilibrium (3). Samples were taken from the lung as nearly as possible synchronously with the samples taken from the aerotonometer.

The following results were obtained.

March 24, 1911. Blood from Dog ♂ 21 kgm. Barometer 755 mm. Hg. Temperature 30°C.

TIME	OBSERVATIONS AND LUNG PRESSURE	INTERVAL IN MINUTES	AIR SAMPLES FROM LUNG AND TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
1.00	Circulation established							
1.04	Sample 1, 6 mm..........	4	2.23	2.19	16.0	18.14	18.23	133
			2.15			18.32		
1.57	Sample 2, 6 mm..........	57	3.50	3.47	25.4	15.61	15.65	114
			3.34			15.68		
2.10	Expired air into tonometer							
2.18	Added 1 cc. of 10 per cent NaF							
2.54	Sample 3, 6 mm..........	114	4.08	4.06	29.7	12.98	13.09	96
			4.04			13.16		
3.00	Aerotonometer sample, 5 mm...................	120		4.09	29.4		12.88	93

April 10, 1911. Dog ♂ 15 kgm. 225 cc. blood. Temperature 30°C. Barometer 755 mm. Air samples from lung and tonometer.

12.05	Circulation established. Expired air into tonometer							
12.30	Lung sample, 7 mm.......	25	3.21	3.46	25	10.81	11.09	82
			3.71			11.37		
12.48	Tonometer sample, 0 mm.	43		4.28	31		12.33	89
1.20	Expired air into tonometer							
1.35	Lung sample, 7 mm.......	90	3.48	3.57	26	8.55	8.58	63
			3.66			8.61		
1.45	Lung sample, 7 mm.......	100	3.54	3.55	26	7.84	7.50	55
			3.55			7.16		
1.50	Tonometer sample, 0 mm.	105		4.82	35		8.10	59

May 12, 1911. Dog ♂ 25 kgm. 225 cc. blood. Temperature, 33°C. Barometer 747 mm.

1.09	Circulation established. Expired air into tonometer							
1.35	Lung sample, 15 mm......	26	2.98	2.81	20	13.52	13.61	99
			2.65			13.71		
1.40	Tonometer sample, 0 mm.	31	3.54	3.48	25	13.80	14.15	100
			3.43			14.51		
1.50	Expired air into tonometer							
2.23	Lung sample, 13 mm......	33	3.94	4.03	28	11.16	11.12	80
			4.12			11.09		
2.28	Tonometer sample, 5 mm...................	38	3.67	3.67	26	12.83	12.78	98
			3.68			12.74		

March 17, 1911. Dog ♂ 20 kgm. Thermostat 27°C. Barometer 756.8 mm.

TIME	OBSERVATIONS AND LUNG PRESSURE	INTERVAL IN MINUTES	AIR SAMPLES FROM LUNG AND TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
12.01	Circulation established.							
12.07	Expired air into tonometer							
12.11½	Lung sample 1, 7 mm.....	10½	3.24 3.52	3.38	25	13.29 13.15	13.22	97
12.15	Added 2 cc. of 10 per cent NaF							
12.19	Lung sample 2, 7 mm....	18	3.45 3.41	3.43	25	13.00 12.97	12.99	96
12.40	Tonometer sample 0 (33′)	39	3.27 3.90	3.58	26	15.03 14.11	14.57	
12.42	Expired air into tonometer							
12.43½	Lung sample 3, 7 mm.....	42½	3.46 3.30	3.38	25	12.30 12.12	12.21	90
1.16	Aeronometer sample, 0 mm (36)...............	70	3.94 3.28	3.61	26	10.64 10.87	10.75	78

These experiments show a general correspondence between the tension of the gases in the lung and in Krogh's tonometer. For example:

April 10, 1911. After 25 minutes interchange of gases in the blood and in the lung and tonometer, the lung showed a CO_2 tension of 25 mm. Krogh's apparatus taken 18 minutes later 31 mm., while the oxygen values were 82 mm. and 89 mm. respectively. In the same experiment an hour later the results were as follows:

	CO_2	O_2
Krogh's..................................	35	59
Lung......................................	26	55

In an experiment of *March 24, 1911*, a sample of gas from the lungs was taken 34 minutes after introducing air into the tonometer, and 6 minutes later a sample was taken from the tonometer. The analysis shows that the lung and tonometer give the same result within the limits of experimental error. In this experiment the lung had been reduced to a physical structure by killing the cells by the addition of sodium fluoride to the blood. Evidently the lung acts as a tonometer independently of any vital activity of the alveolar cells.

Many difficulties were encountered in using Krogh's aerotonometer. Especially was it found difficult to obtain a good film of blood since the blood ran down the tube in streaks. Another difficulty was that only a small quantity of gas was available for analysis.

In the final series of experiments a new type of aerotonometer was employed, designed by Doctor Brodie, which affords plenty of gas for duplicate analyses and in which the blood is spread out in an even film, which is constantly renewed by the rotation of the tonometer (4). This apparatus is shown connected in parallel with the lung in the figure.

The equilibrium time of this tonometer has been ascertained to be ten minutes with a circulation time of once every two minutes, which is comparable to the rate of flow through the lungs in living animals to fifteen minutes in a circulation time of once every three or four minutes. The following results were obtained:

December 29, 1911

TIME	OBSERVATIONS	LUNG PRESSURE	TEMPERATURE	INTERVAL IN MINUTES	AIR SAMPLES FROM LUNG AND TONOMETER					
					CO_2			O_2		
					Analysis	Mean	Tension	Analysis	Mean	Tension
		mm.	deg. C.		per cent	per cent	mm.	per cent	per cent	mm.
10.40½	Circulation established									
10.42	Lung sample.........	0	17	1½	0.82 0.62	0.72	5	17.00 17.28	17.14	127
10.49	Tonometer sample...	0	17	8½	1.04 0.76	0.90	7	17.63 17.53	17.58	130
10.51	New air introduced into tonometer									
11.06	Lung sample.........	0	17	15	1.12 1.00	1.06	8	16.28 16.36	16.32	121
11.13	Tonometer sample...	0	17	22	1.18 1.10	1.14	8	17.88 17.76	17.82	132

The blood in this experiment was obtained from a dog used in the morning. The blood and thorax had been kept in a refrigerator.

April 12, 1912. Lung taken from a dog on April 9. Saline was run in as the animal was bled. Subjected to artificial ventilation with formalin vapor for 5 hours. Lungs kept on ice. Blood taken from dog about 200 cc.

TIME	OBSERVATIONS	PRESSURE OF GAS IN LUNG	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNGS AND TONOMETER: CO_2 Analysis	CO_2 Mean	CO_2 Tension	O_2 Analysis	O_2 Mean	O_2 Tension	RATE OF FLOW
		mm.	*deg. C.*		*per cent*	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*	*cc./min.*
3.51	Circulation established										
4.01	Lung sample...	9	21.5	10	0.93 1.07	1.00	8	18.50 18.38	18.44	137	
4.06	Tonometer sample.......	−5	21.5	15	1.55 1.47	1.51	11	17.19 17.09	17.14	125	10
4.08	Expired air into tonometer										
4.19	Lung sample...	9	20.2	11	1.32 1.16	1.24	9	18.41 18.37	18.39	137	
4.23	Tonometer sample.......	5	20.2	15	0.77 0.55	0.66	5	18.69 18.63	18.66	137	60

April 20, 1912. Fresh lung of cat; about 100 cc. of cat's blood made up to 200 cc. with 0.85 per cent saline. Rate of flow in lung 100 cc./min. Circulation time 1 every 2 minutes. Rate of flow in tonometer 60. cc./min. Circulation time 1 every 3.3 minutes. Barometer 755.

TIME	OBSERVATIONS	PRESSURE OF GAS IN LUNG	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNGS AND TONOMETER: CO_2 Analysis	CO_2 Mean	CO_2 Tension	O_2 Analysis	O_2 Mean	O_2 Tension
		mm.	*deg. C.*		*per cent*	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
5.45	Circulation established									
5.55	Lung 1...............	2	16.5	10	1.56 1.48	1.52	11	18.18 18.22	18.20	135
6.05	Tonometer 1..........	−5	15.8	20	1.25 1.31	1.28	10	18.98 18.91	18.95	140
6.06	Air into lungs and tonometer									
6.21	Lung 2...............	15	15.5	15	0.80 0.69	0.75	6	18.88 18.82	18.85	143
6.23	Tonometer 2..........	+5	15.3	16	1.05 0.92	0.99	7	19.24 19.16	19.20	142
6.28	Lung 3...............	7	15.3	22	0.55 0.54	0.55	4	18.97 18.92	18.94	142
6.29	Tonometer 3..........	+8	15.2	22	0.60 0.53	0.56	4	18.74 18.69	18.72	137

In these later experiments the carbon dioxide tension was very low. In the following experiments the blood was saturated with CO_2 before it was introduced into the circulation.

January 7, 1913. 190 cc. of blood from a dog 7 kgm. Barometer 757. Aerotonometer revolving 36/min. Arterial pressure 60/mm.

TIME	OBSERVATIONS	PRESSURE OF GAS IN LUNG	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNG AND TONOMETER					
					CO_2			O_2		
					Analysis	Mean	Tension	Analysis	Mean	Tension
		mm.	deg. C.		per cent	per cent	mm.	per cent	per cent	mm.
1.32	Circulation established									
	Rate of flow: Tonometer 48 cc./min.									
	Rate of flow: Lungs 34 cc./min.									
1.47	Lung sample 1........	10.5	18.3	15	5.93	5.96	44.8	15.74	15.60	117.0
					6.00			15.49		
1.51	Tonometer sample 1...	1.0	18.3	19	6.20	6.33	46.8	16.04	16.00	118.4
					6.46			15.96		
1.53	Expired air into tonometer									
1.54	Expired air into lungs									
2.08	Lung sample 2........	10.5	18.2	14	3.69	3.78	28.4	17.30	17.32	130.2
					3.87			17.34		
2.09	Tonometer sample 2..	1.0	18.2	16	3.75	3.78	28.0	16.99	16.92	125.3
					3.81			16.84		
2.25	Expired air into lungs and tonometer									
2.40	Lung sample 3........	10.5	18.6	15	2.83	2.91	21.9	18.12	18.09	135.9
					2.98			18.05		
2.44	Tonometer sample 3..	1.0	18.6	19	3.12	3.11	23.0	17.57	17.64	130.5
					3.11			17.71		
	Rate of flow: Tonometer 50 cc./min.									
	Rate of flow: Lungs 20 cc./min.									
2.50	Expired air into tonometer									
2.51	Expired air into lungs									
3.06	Lung sample 4........	+28.0	17.8	15	2.00	1.98	15.2	18.42	18.35	141.4
					1.96			18.29		
3.09	Tonometer sample 4...	− 1.0	17.8	19	1.92	1.97	14.6	18.10	18.23	135.1
					2.02			18.36		

DISCUSSION

In the experiment of December 29, after the gases in the blood had been in contact with the gases in the tonometer and with the gases contained in the freshly excised lung for a period of from ten to twenty minutes, a close correspondence in the gaseous tension was obtained. In the experiment of April 12, a similar agreement was obtained after allowing an interchange of gases for fifteen minutes, but in this instance the alveolar cells of the lung had been killed by ventilating the lung previously with formalin vapor for several hours. The experiment of April 20 shows a close correspondence after fifteen minutes exposure.

Four sets of satisfactorily corresponding tensions were obtained in the experiment of January 7. The gases in the lung undoubtedly reach equilibrium in about one minute but as ten to fifteen minutes must be allowed to be confident that equilibrium has been attained in the Brodie tonometer, the samples were taken at longer intervals.

These experiments show that the lung acts as a tonometer either freshly excised or after having been killed by chemicals.

The accuracy of the determination of gaseous tension as shown by the duplicate analyses does not permit a comparison beyond a decimal point. In the earlier experiments an error of 5 per cent must be allowed but after long practice duplicate analyses were obtained which showed a variation of not more than $\pm$ 0.05.

In making use of an excised lung to determine the gaseous tension of the blood of a second animal, it was found that the blood clotted much more readily in lungs which had been fixed by formalin than it did in fresh lungs. Moreover in using a cat's lung to determine the tension of dog's blood, clotting was encountered. The best results were obtained when using a puppy's lung to determine the tension of dog's blood.

CONCLUSIONS

The excised lung, either freshly prepared or after treatment with sodium fluoride or formalin, when perfused with defibrinated blood, acts solely as a physical surface and may be used as an aerotonometer. It affords sufficient surface so that equilibrium between the gases in the blood and the gases in the alveoli is attained in at most one and one-half minutes, which is the time required to take a sample.

It was the late Prof. T. G. Brodie who originated the conception that the lung might be used as an aerotonometer and his kindly counsel in

directing my work has been the source of any value that the research may contain.

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ON THE HEAT LIBERATED BY THE BEATING HEART

Second Communication

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Received for publication July 19, 1918

Having shown in a former communication (1) that the chelonian heart during its beat undergoes a measurable change of temperature, it seemed desirable to obtain more objective records of this change, for it will be remembered that the method of registration of the galvanometer response to the rise and fall of temperature consisted of a mechanical lever operated by the observer. If photographic registration could be substituted for the hand-operated recorder this subjective element could be removed.

The use of the Einthoven instrument on account of its fitness for photographic registration had already been considered, but that instrument also has its disadvantages as will appear in the following.

The fiber I happen to have is a gilded quartz fiber of 5400 ohms resistance. The galvanometer is the small Edelmann model. To give an idea of its availability for recording thermo-electric effects from a turtle's heart beating say, at a rate of five per minute, the following observations will serve.

With the tension of the thread set at 15, the calibrating current produced a deflection of 5 mm. of scale, with the tension set at 16, the deflection to the same current was increased to 22.5 mm. of scale. The periods of the instrument at the two tensions of the thread, however, were 0.7 and 2.7 seconds respectively. The calibrating current was of constant strength, permitting 4.6×10^{-9} ampere to pass through the galvanometer. With the clasp-thermopile in series with the galvanometer the difference of temperature between the warm and cold junctions necessary to create an amperage equal to the calibrating current would have to amount to nearly 0.0156°C. The difference of temperature that could be registered by 1 mm. deflection of the galvanometer

thread set at the two tensions named above would then be nearly 0.003° and 0.0007° respectively.[1]

In my former study it was shown that a rise of about 0.0013° may be expected per beat of the turtle's ventricle. With the tension of the thread in the galvanometer set at the greater of the two sensibilities one would expect a deflection of 2 mm. per beat, provided further that the duration of the beats is at least twice the period of the galvanometer. The period being 2.7 seconds the duration of beat should be reduced to 5.4 seconds. If the duration of the beat should happen to be 5 instead of 5.4 seconds it is clear that a slight increase in tension on the thread will bring its period within the limits prescribed without reducing the sensibility greatly.

[1] For this calculation the reader is referred to the formula given on page 424 of my first communication (1). The derivation of that formula was not given there. For purposes of checking up results it is here added:

Let I be the strength of thermo-electric current produced by the thermopile in use when a difference of 1°C. obtains between the two sets of junctions and when the current passes through the thermopile (of r_1 resistance) and the galvanometer (of r_2 resistance) in series. Then when the calibrating current (in the present case 4.6×10^{-9} amp.) produces a deflection of m divisions of scale the rise of temperature necessary to give an equal deflection will be i/I, where i represents the amperage of the calibrating current. This value then divided by the number of scale divisions deflection to the calibrating current, m, will give the difference of temperature represented by 1 scale division deflection, Δt.

In the present experiments I has the value, $\frac{30 \times 53 \times 10^{-6}}{5400 + 11.32}$, and i has the value, 4.6×10^{-9}. Where i yields 22.5 as a value for m, Δt equals 0.00069; with 5 as a value for m, Δt equals 0.003°C.

Combining the operations into one generalized equation we have the formula,

$$\Delta t = \frac{r_1 + r_2}{n \cdot p \cdot m} \cdot i$$

as given in my former paper, in which n represents the number of elements and p the thermo-electric power of one element, in the thermopile used.

To complete the account it should be added that the calibrating current was derived from a normal cell of 1.0187 volts with a constant resistance of 38,220 ohms connected in series to a shunt of 1 ohm and the galvanometer of 5400 ohms arranged in parallel. The total resistance of the circuit thus remains practically at 38,220 ohms and the total amperage is 2.5×10^{-5}. The strength of current passing through the galvanometer thus is tot. amp. times the product of the reciprocals of the parallel resistances, or $i = 4.6 \times 10^{-9}$ ampere.

Further it should be stated that the deflections of the galvanometer are taken to be a linear function of the strength of current passing which for the ranges of current employed is practically the case.

With the foregoing discussion as to what may be expected of the Einthoven instrument in mind, I will now proceed to a brief description of the experiments and their results. For details of method and apparatus not here given the reader is referred to the former study.

Experiment of March 15, 1918. A turtle's heart was suspended in the moist chamber with the clasp-thermopile in place. The action of the heart on the thermopile junctions was observed for some minutes. No change in position between heart wall and junctions took place and it was assumed that this condition continued during the experiment. Indeed one can be certain, after some experience, from the character of the galvanometer deflections and especially from their photographs just when the contacts remain in ideal relation. The moist chamber having been supplied with ice the temperature soon fell to about 12.5°C. and remained there during the taking of records. The rate of the beating ventricle was thereby slowed down to about 12 beats per minute. The mechanical movements of the ventricle walls were recorded by an isometric lever that had been carefully calibrated. A time trace was provided for by a Jaquet marking fifths of a second. The sensibility of the galvanometer was noted just before and after each photograph taken. The rate of the moving film and its length permitted a record of two or three complete heart beats to the photograph. Memoranda and analysis of two of the records so taken may be here reproduced.

Photo-record 1. Calibrating current just before gives 15 mm., just after gives 12.5 mm. deflection of thread. Distance of optical projection, 119 cm. Distance of recording tip of mechanical lever from camera shutter, 13 cm. Measurement of the curves in the photograph shows a maximum excursion of the mechanical lever of 13.0 mm.; a maximum deflection of the galvanometer thread of 1.9 mm., which is monophasic and single for the whole of a cardiac cycle and begins nearly a second after the beginning of the rise of the myogram curve. The duration of a single heart beat is 5 seconds, of the systole (ascending limb of myogram) 2.2 seconds, diastole and pause 2.8 seconds. The measurements are the same for all the heart beats in the record.

Photo-record 2. Deflection of galvanometer to calibrating current remains constant, 15 mm. of scale. Other conditions the same as for the preceding record. Measurements from the photograph: maximum excursion of mechanical lever, 13.5 mm.; maximum deflection of thread, 2.1 mm.; initial rise of galvanometer deflection 0.5 second after initial rise of mechanical lever; duration of each beat, 5.4 seconds; of systole, 2.0 seconds; of diastole and pause, 3.4 seconds.

At the end of the experiment the ventricle free from large vessels, auricles and blood was found to weigh 3.6 grams.

Calculations. From the calibration data of the mechanical lever (not here reproduced) and from the data of the observed optical projection one can deter-

mine the actual shortening of the ventricle wall and the maximum tension developed during each heart beat. From the data on the electrical and thermo-electrical quantities involved, as given above, one can calculate the changes of temperature represented by the galvanometer deflections.

The results of the corrections for the mechanical curve show an actual maximum shortening of the muscle of 1.18 mm. and a maximum tension developed during each systole of 13.7 grams for each beat in the first record and 14.3 grams for each beat in the second record. The sensitivity of the galvanometer was not the same for both records,—not more than 13.75 mm. deflection can be allowed for the first record, as against 15 in the second. So while there appears to be considerable difference in the rise of temperature in the two records actual calculation, according to the method given above, shows a rise of temperature during each heart beat in the first record to be about 0.00215°C. and in the second record to

ERRATA

[To be inserted between pp. 158 and 159, Vol. xlvii.]

Page 159, line 15, for "0.0007" read ".0018,"
Page 160, line 4, for the sentence beginning here read, "The heat produced was .83 × .00156, or .0013 calorie per gram ventricle."
Page 163, line 19, for "0.00048, 0.00052 and 0.00073", read ".0011, .0013 and .0018."
line 21, for "0.00062" read, ".00155"
line 30, for "1.3" and "1.67" read "3.2" and "4.5."
Page 164, last line, for "well within" read "nearer the greater of."

appropriate to the shadows of the other recording levers.

The first photo-record in this experiment was not brought to a successful finish. The second was more successful and the data for this are as follows:

Photo-record 2. Initial tension in heart-bag, that is, diastolic pressure, 25 cm. water. Temperature of moist chamber, 16.5°C. Deflection of galvanometer thread to calibrating current, 20 mm. of scale.

In the photograph the duration of the heart beat is about 9.6 seconds (partial block due to slitting ventricular septum?). The duration of the systole, that is of the ascending limb of the manometer lever, is about 2 seconds. The manometer lever shows in each case an excursion of 3 mm. or a rise of tension during systole of 6 cm. water-column.

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Experiment of May 3, 1918. In this experiment the method and instrumentarium are all the same except that a Rohde (2) heart-bag was used instead of the isometric muscle lever to record the mechanical events. The bag fixed at the end of a cannula was inserted into the ventricle chamber through the aortic arches, the intervascular septum and the ventricular septum having been previously slit down sufficiently to make a single passage way and chamber. The bag was made to communicate with a Hürthle membrane manometer. This manometer was calibrated just before the experiment so that the excursions of the recording tip could be converted into terms of a column of water pressure. In this experiment 0.5 mm. excursion of the lever tip was equal to a difference of 10 mm. change in the height of a column of water. The recording tip of the manometer lever was so adjusted as to have its shadow fall across the aperture in the camera in a position appropriate to the shadows of the other recording levers.

The first photo-record in this experiment was not brought to a successful finish. The second was more successful and the data for this are as follows:

Photo-record 2. Initial tension in heart-bag, that is, diastolic pressure, 25 cm. water. Temperature of moist chamber, 16.5°C. Deflection of galvanometer thread to calibrating current, 20 mm. of scale.

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The galvanometer thread shows a deflection of 2 mm. for each heart beat which I calculate to represent a rise of temperature of 0.00156°C. The heart was not weighed in this experiment but since the turtles used were nearly the same size one can make an estimate of the ventricle's weight. If that is taken as 3.6 grams then the heat production was 0.000526 calorie; if the weight is taken as 3.0 grams then the heat production was 0.000531 calorie per gram ventricle per beat.

Explanatory note to the figures in the folder. Before passing on to any general discussion a word explanatory to the figures as they appear in the attached folder should be added. The figures in each case should be read from left to right. The nethermost tracing in each figure is that of the time marking fifths of seconds. The heavier of the two tracings in the body of each figure is that of the mechanical lever (whose ascending limb is caused by the systole of the beat), the lighter one will be recognized as that of the galvanometer thread.

Figure 1 is photo-record 1 of the experiment of March 15. Here the mechanical curve represents an isometric muscle lever attached to the frenum of the heart.

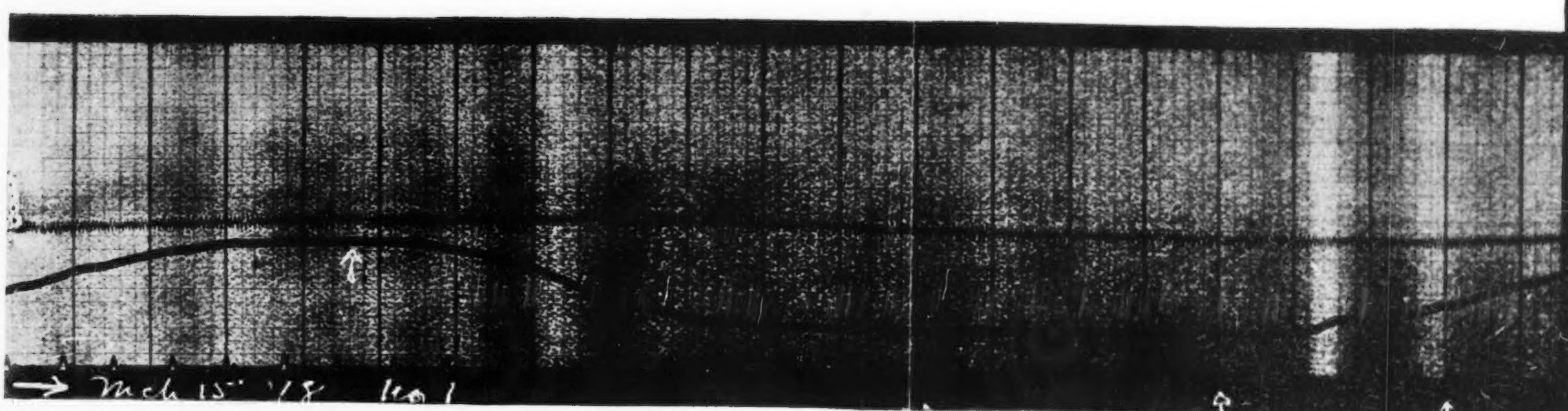
Figure 2 is photo-record 2 of the experiment of May 3. Here the mechanical curve represents the excursions of a Hürthle manometer lever, hence the intraventricular pressure changes or the "pulse pressure." The ascending limb again represents the systole of the beat. In this experiment while the ascending limb of the manometer lever occurs during the heart's systole, the initial rise of the curve of this lever in the photo-records cannot be taken as the point of beginning rise of tension. The initial tension was fixed at 25 cm. The early stages of systole during which this amount of tension is developed receive no indication in the record. No definite statement therefore can be made about the duration of the systole.

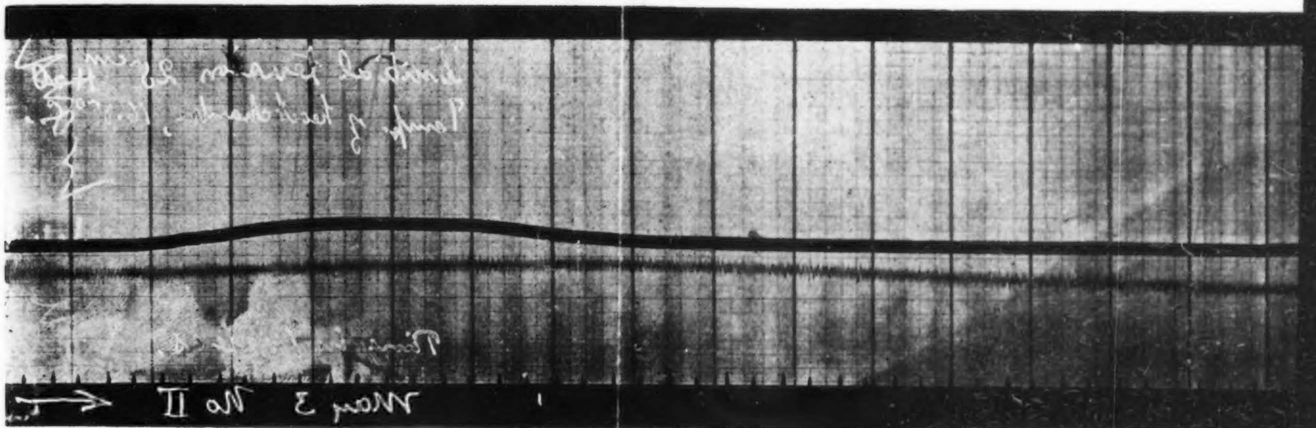
The fine horizontal and vertical straight lines found in the photo-records will aid the eye in comparing synchronous events at any point desired.

DISCUSSION

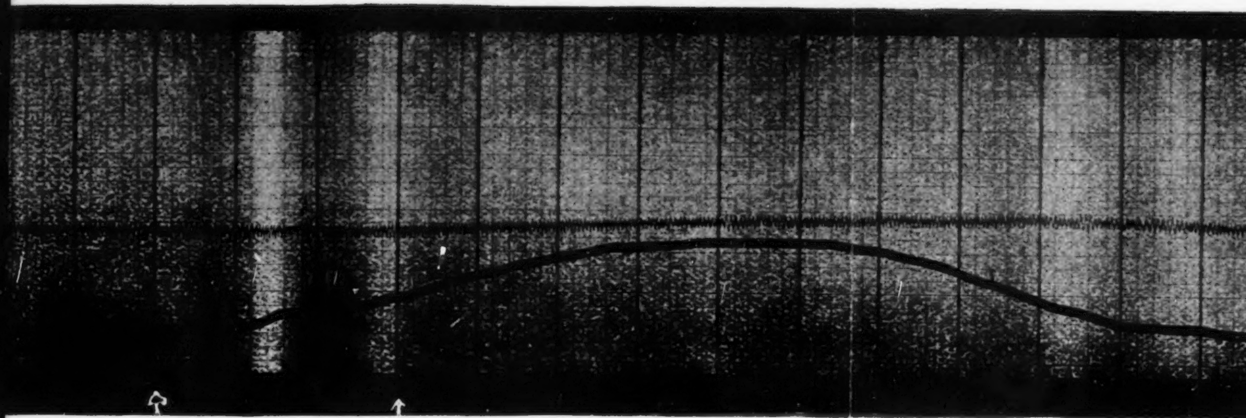
1. The initial heat production in the cardiac cycle. The temporal relations between the initial rise of temperature and the initial shortening of the heart musculature involves one of the most important theoretical points and may be considered first.

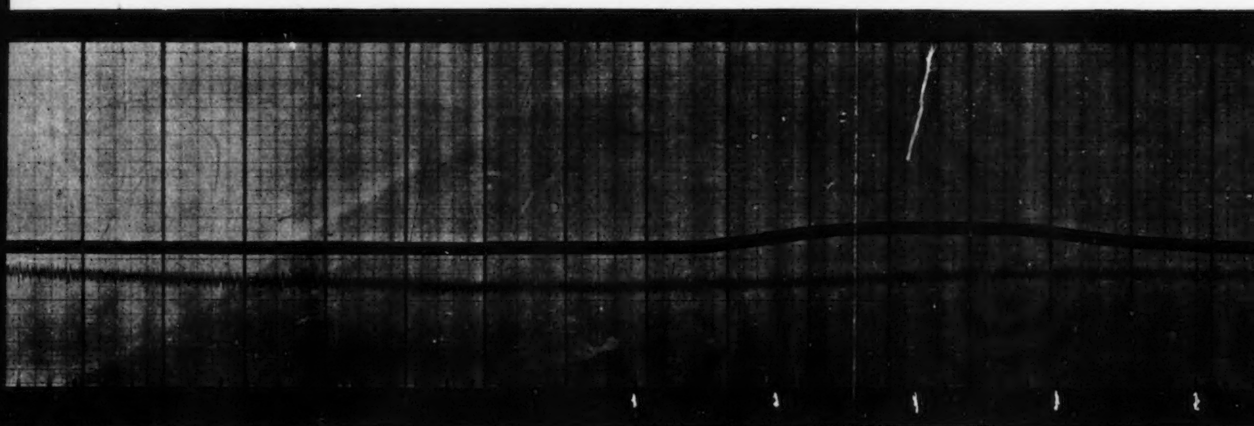
The time required for the conduction of heat from the tissue to the thermal junctions of the recording apparatus has already been discussed in my former paper. It was there pointed out that the cooled and blocked turtle's ventricle was a specially suitable preparation for the study of heat production in muscle. It was pointed out that the probably long latent period to the inner stimulus (estimated to be from 0.3 to 0.5 of a second) gave an abundance of time for the conduction of heat if any measureable amount were formed during this period. In that study no evidence appeared of heat production during the



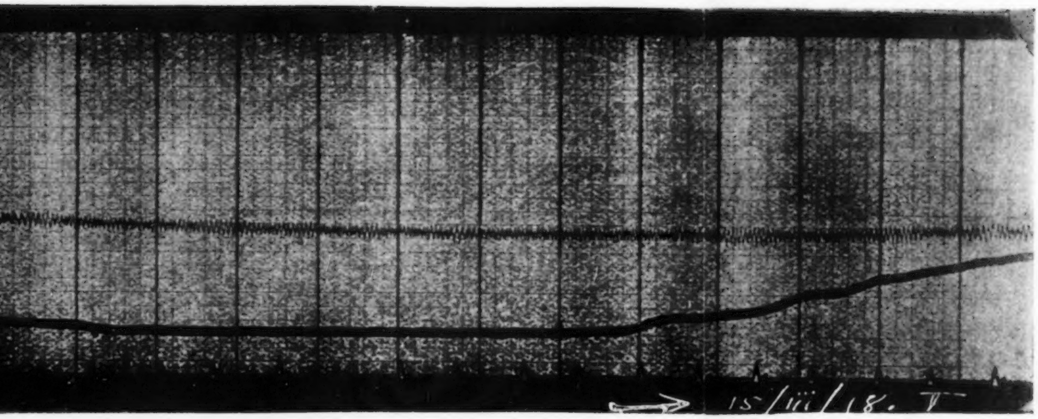


NOTE. The upper figure is referred to in the text as figure





The upper figure is referred to in the text as figure 1, the lower as figure 2. **See page 160 for explanatory note.**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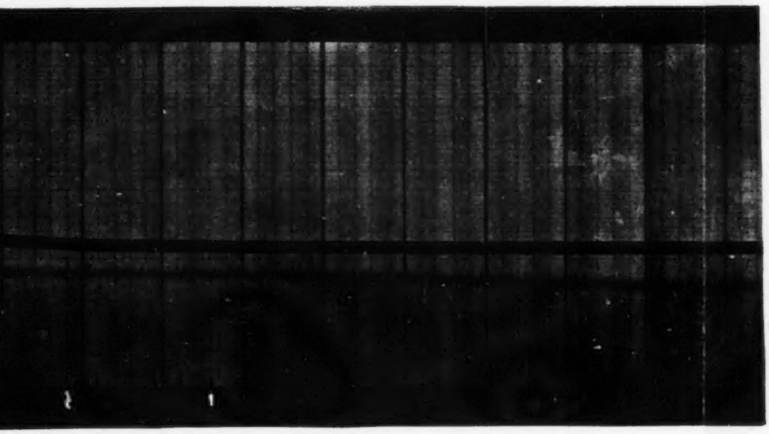

latent period of the muscle contraction or before actual rise of tension occurred in the muscle. The records obtained in the present study, where an isometric lever of small initial tension was used, are similar in this respect to those of the former study. No evidence of pre-systolic heat production appears. In the experiment of May 3, however, where a high initial tension was placed upon the heart-walls it would appear at first sight that heat is liberated during the pre-systolic period (fig. 2 of the folder). Here seems to be a contradiction. But when one reflects upon the conditions of the two experiments this contradiction disappears.

In the first place one must consider the difference in the initial tensions placed upon the muscle in the two experiments. In the first case the initial tension was just enough to balance the weight of the heart, in the second case no weight was needed to balance the weight of the heart but nevertheless its walls were kept distended by the pressure of a column of water 25 cm. in height during diastole. This initial tension was selected by a preliminary trial which showed that no greater tension could be placed on the relaxed muscle and still allow one to get a recordable response of the manometer lever during systole. The tension allowed an increase during systole of 6 cm. in the water column. But before the height of the column could be increased the heart wall had to develop an internal tension from 0 to 25 cm. of water. It is during this period that the record shows no mechanical response to the rising tension. It thus fails to record the early stages of systole. The ascending limb of the galvanometer response in the photograph therefore is initiated before that of the mechanical lever, not because heat production begins before rise of muscular tension but because the mechanical lever fails to record the initial rise of tension.

It may be stated that the experiment of May 3 was only the beginning of a series in a study on the relation of heat quantities and tensions in muscle contractions.

One other important correction should be kept in mind in comparing the mechanical and thermal curves in the photographs. In both experiments the latency of the former is doubtless much greater than the latency of the latter. The latencies have not been actually measured but if their ratios are as we may expect, then supposing the two events to arise within the tissue simultaneously, the mechanical curve would have its beginning in the photograph at a point subsequent to that of the thermal curve by difference of time equal to the difference of their latencies. Now the reverse order of events appears in the

records unless one increases the latency of the mechanical event artificially, that is, by raising the initial tension to a point approximating the maximum tension developed.

Summing up, I conclude that the thread galvanometer gives no evidence that heat is produced in the turtle's heart muscle during the pre-systolic period, or during the period that may be called the latent period of contraction or before there occurs within the tissue a rise of tension. This agrees with the conclusion arrived at, using the Paschen galvanometer.

2. *Velocity of heat production.* As to rate at which heat is liberated at successive intervals of time the records from the Einthoven galvanometer are unsatisfactory. This is due to the small total excursion of the thread which, while great enough to admit of accurate measurement of total rise of temperature over a period of two seconds, does not admit of a measurement of partial rises over periods say, of tenth of seconds. As far as one can judge by mere inspection however the character of the curves in this study is quite similar to that of the curve obtained from the Paschen galvanometer. The rise of temperature proceeds at first slowly then with increasing velocity and finally changes again to a slower rate just before reaching the maximum rise. Indeed the turning point in the curve may be looked upon as being due not to an abrupt cessation of heat liberation but rather to a reduction in the velocity of its liberation to a point where it no longer balances the loss of heat by conduction, etc. For the time of return to null point, that is, of cooling exceeds the period of the galvanometer.

3. *Maximal, mechanical and heat effects.* It will be noted that the maximum point in the galvanometer curve in the photographs occurs at a point subsequent to the maximum point of the mechanical curve. This delay in the galvanometer maximum can only be explained if we take into consideration the period of the galvanometer. This point was pretty fully discussed in my former paper and need not be gone into again. It is a noteworthy fact however that the delay in the two maxima is in the present study, as in the former, equal nearly to the period of the galvanometer. Parenthetically it may be well to point out again the fact that one has not gained anything in the way of period, the Einthoven being as slow almost in its period as the Paschen. In the curves of figure 1, counting between mid-points of the crests, the delay is a little more than one second; in those of figure 2, a little less than two seconds. Turning to the protocols one notes a sensitivity of 13.7 mm. for the deflection in the curves of figure 1 as against 20 mm.

for the deflections in figure 2, indicating a greater tension of thread therefore a smaller period for the former than for the latter. From the test of the relation between tension and period (see above) one would expect the delays just as we find them in the photographs if the maximum temperature of the muscle was reached just at or slightly before the maximum point of tension.[2]

I conclude therefore that the maximum rate of heat production occurs during the latter half of systole and that the highest temperature of the muscle of the heart is reached during the time of highest internal muscular tension.

4. The total rise of temperature and total heat production during the heart beat. In what was considered the most satisfactory experiment with the Paschen galvanometer the total rise of temperature per heart beat was found to be 0.00132°C. In the present study as already stated this number is 0.00156 and 0.00218 respectively for the two experiments. Assuming no loss of heat by conduction, evaporation, etc., before the rise of temperature is registered by the galvanometer, these figures when converted into gram-calories per gram ventricle are 0.00048, 0.00052 and 0.00073 respectively. Taking the mean of the last two figures, one may express the results of the present study with the Einthoven galvanometer as amounting to 0.00062 calorie of heat liberated per gram of ventricle per beat.

The heat equivalent of Vernon's CO_2 output of surviving turtle's hearts under similar conditions (3) I calculate to be 0.00061 calorie per beat per gram of muscle. It is assumed that the CO_2 arises from the complete oxidation of a definite quantity, the molecular equivalent, of dextrose, which in this case amounts to 1.6×10^{-7} gram.

Assuming also that the heat liberated in my preparations of turtle hearts had its origin in the oxidation of dextrose, the amounts required in the two sets of experiments would be 1.3×10^{-7} and 1.67×10^{-7} gram respectively per gram ventricle per beat.

Turning to actual determinations of sugar consumption by the surviving heart I have at hand only those made upon the mammalian organ at temperatures near 37°C.

In three experiments with the heart of the cat Müller (4) found from 0.0253 to 0.0279 gram of dextrose to disappear per hour. No weights

[2] The latency of the mechanical or rather the difference between the latencies of the mechanical and thermal levers, should be taken account of here. But that could only be a question of a hundredth of a second or so and would not affect the result in this case.

of the hearts or rate of beat are given. But if a weight of 12 grams is assigned to the former and a rate of 120 to the latter a consumption of about 3×10^{-7} gram per gram muscle per beat is indicated. Among the experiments of Locke and Rosenheim (5) there is one in which all the desirable data are given. It is experiment 2, a rabbit heart of 6.25 grams, rate of beat from 156 to 205 per minute. Taking 180 as the mean rate the dextrose consumption per gram of heart per beat appears to be 1.3×10^{-7} gram. Rohde (6) found a consumption of "10 to 40 mg." sugar per gram of cat's heart, indicating a utilization per beat similar to the quantity in Müller's cases, namely 3×10^{-7} gram. Taking the mean of the six determinations of Underhill and Prince (7) on hearts of "well fed" rabbits whose rates of beat "varied from 74 to 144 per minute," the consumption per beat per gram heart is 2.5×10^{-7} gram dextrose.

Clarke (8) who paid especial attention to the prevention of loss of sugar through the action of bacteria and to the promotion of its utilization by the muscle through the action of the pancreas, obtained an interesting series of variation in dextrose consumption in the consecutive hours of the perfusion. When we are once in a position to consider the total energy output of the heart under its various factors of regulation this data will be of special significance. For the present the mean dextrose utilization in these determinations on hearts (of the dog in this case), when perfused without the pancreas in the path of circulation, may be stated as about 0.175 mgm. per gram of heart per hour. With the pancreas in the path of circulation the mean number is about 0.4 mgm. Assuming the rate to have been on the average 100 beats per minute (it is stated that the hearts beat vigorously) the utilization per beat appears to be about 0.3×10^{-7} and 0.7×10^{-7} gram respectively.

Taken all in all, then, the sugar consumption of the mammalian heart for the most part may be put between 0.3×10^{-7} and 3.0×10^{-7} gram per gram muscle per beat. The quantities of dextrose which are equivalent to the quantities of heat observed to be liberated by the beating chelonian heart lie well within these limits.

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